PHARMACOLOGY/TOXICOLOGY COVER SHEET

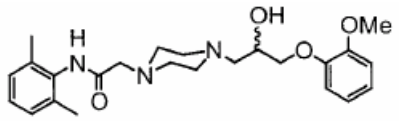
NDA number:21-526
Review number: 1
Sequence number/date/type of submission: NDA
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: CVT Therapeutics
Manufacturer for drug substance :

Reviewer name: Elizabeth A. Hausner, D.V.M.
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Review completion date: August 27, 2003
Drug:

Trade name: Ranexa
Generic name (list alphabetically): ranolazine
Code name: RS-43285-193, RS-43285-003, CVT-303, RAN D, Ran4
Chemical name:

IUPAC:	N-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazinyl}acetamide
CAS[1]:	1-piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-
Other:	(±)-4-[2-hydroxy-3-(*o*-methoxyphenoxy)propyl]-1-piperazineaceto-2',6'-xylidide

CAS registry number: 95635-55-5
Mole file number:
Molecular formula/molecular weight: $C_{24}H_{33}N_3O_4$/427.54
Structure:



Ranolazine

Relevant INDs/NDAs/DMFs: IND 43,735
Drug class: anti-anginal
Indication: angina
Clinical formulation: sustained release tablets of 375 and 500 mg. Tablet: methacrylic acid copolymer, microcrystalline cellulose, hydroxypropyl methylcellulose, sodium hydroxide, magnesium stearate. Coatings: titanium dioxide, polyethylene glycol, FD&C blue # 2 lake or FD&C yellow #6 lake, polysorbate 80, carnauba wax. Maximum recommended dose of 1000 mg b.i.d.
Route of administration: oral
Proposed use: angina for those patients in whom all other anti-anginals are inadequate or not tolerated
Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: Preclinically ranolazine has been shown to interact with cardiac ion channels. Approximately 7 of the known major metabolites have also been shown to interact with cardiac ion channels including Ikr. There is no projected margin of safety between plasma levels in animals where various adverse effects have appeared and the plasma levels measured in humans. Approvability depends upon the clinically demonstrated risk:benefit ratio.

B. Recommendation for Nonclinical Studies: 1)Exploration of the long-term effects of ranolazine upon the pigmented structures of the eye. 2) Clarification of the apparent reproductive liability of the drug.

C. Recommendations on Labeling: Under "Description": There is insufficient information to warrant the statement that ranolazine "works primarily through partial inhibition of fatty acid oxidation and is pharmacologically unrelated to calcium channel blockers, beta blockers and nitrates."
The "Mechanism of Action" section should be restated to reflect the uncertainty of the mechanism of action. The paragraph regarding in vitro electrophysiology should be removed.

"Carcinogenesis, Mutagenesis, Impairment of Fertility": The sponsor's statement should be changed to read that ranolazine has shown detrimental effects upon male fertility at doses of 300 mg/kg/day (1800 mg/m^2 or 1.5 times the MRHD of 1200 mg/m^2 on a surface area basis as stated by the sponsor Vol. 2, p 7). Ranolazine showed dose dependent embryotoxicity in rabbits at doses of 6 m/kg (74 mg/m^2, 0.06X MRHD), 45(555mg/m^2 0.5X MRHD)and 150 mg/kg(1850 mg/m^2, 1.5x MRHD).

"Pregnancy—Category C" The sponsor's first two sentences in this section should be removed and replaced with a statement that in rats, there is evidence of skeletal malformations (sternebrae, pelvic bones and cranial ossification) at doses 5mg/kg (30 mg/m^2 or 0.03X MRHD), 40 mg/kg (247mg/m^2 or 0.2X MRHD) and 400 mg/kg/day (2467 mg/m^2 or 2X MRHD) .

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Safety pharmacology was presented for cardiovascular, gastrointestinal, nervous and pulmonary systems. The cardiovascular safety study showed that cumulatively increasing doses of intravenous ranolazine caused a deterioration in cardiac function manifested as decreased cardiac output, decreased contractile force and decreased left ventricular systolic pressure which could reflect direct cardiac toxicity or increased afterload. Left ventricular minute work was also decreased while total peripheral resistance was increased, suggesting that afterload indirectly hurt cardiac performance. No ECG data was provided. The sponsor was asked by telephone to provide the ECG data. July 2, 2003 the sponsor

replied verbally to this request and said that the ECG data was unavailable and could not be produced. The sponsor's estimate of total cumulative exposure for the 2 doses where these effects were most prominent showed that the plasma concentration was within the range seen clinically.

In a July 31, 2003 telecon with representatives from CV Therapeutics, Drs Koerner and Hausner requested the ECG data for the dog toxicology studies. August 18, 2003 in a telephone call, Maragaret Dillon of CV Therapeutics informed this reviewer that while the ECG data had been located, it had not been analyzed. She estimated that it would take some 6 months of work to quantitate the various ECG intervals. After consultation with Drs Koerner and Gordon, it was decided to forgo review of the dog ECG data. This was communicated back to the sponsor later on 8/18/03.

A variety of in vitro studies indicate that ranolazine has the ability to interact with cardiac ion channels. Lacking informative ECG analyses, this creates a concern for the drug's potential to alter repolarization.

Central sedation was noted in several of the CNS assessments. Neurologic deficits were identified at doses where sedation was not apparent. Ranolazine treatment also contributed to stress-induced hyperphagia, indicating an effect on the hypothalamic-pituitary-adrenal axis. This was reinforced in the general toxicology studies and several special toxicology studies that specifically examined the effect of ranolazine on the adrenal gland (see below).

The gastrointestinal safety study lacked a positive control but was suggestive of a mild delay in GI transit.

Consistent across toxicology studies and species were clinical signs of sedation and salivation. Signs reported primarily in rodents included hunching, piloerection, dyspnea or tachypnea, ataxia, tremors and convulsions. Dogs also showed ataxia, respiratory signs, tremors, thrashing and convulsions. There seemed to be a decreased incidence of signs with prolonged dosing. The sponsor suggested hypotension to explain the signs but it is unclear that hypotension could produce all of the signs reported. The safety pharmacology studies also reported neurologic effects in rodent species at doses for which we do not have information regarding blood pressure. Doses used in the nonclinical studies were limited by the neurologic signs. The highest doses used produced only low multiples of human exposure. Adverse effects in animals were found at plasma drug levels at or below those achieved in humans.

The adrenal gland was identified as a target organ. Special studies showed both acute and chronic effects of ranolazine on the HPA axis. Two oral doses of ranolazine depressed both basal and post-stress ACTH levels as well as corticosterone in plasma and the adrenal gland. All these parameters increased in response to stressors but not to the extent of the vehicle-treated animals. There were some inconsistencies between studies. However, dose-related increases in adrenal weight were seen in both dogs and rats. Histological changes such as vacuolation of the zona fasiculata repeated across studies. While the neurologic effects and to some extent the dermal effects reported in one dog

study are relatively non-specific, opioids have been shown to affect the HPA axis both acutely and chronically. This fact and receptor binding studies suggest that either parent compound or one of the metabolites may have opioid receptor activity. Or, this may be indirect support of the sponsor's proposed mechanism of action (See Summary of Special Toxicology Studies).

Reproductive and developmental toxicology studies indicated adverse effects upon fertility in both sexes. Furthermore, general toxicology studies in both rodents and dogs showed 1) dose-related increases in pituitary size in female rats (1 year study) 2) dose related decrease in absolute and normalized uterine weight in dogs in both 3-month and 12-month studies 3)in males, an increase in absolute and normalized testicular weight was seen in 3 and 12-month studies 4) in the 1 year study, the prostate weight was increased at all doses while testicular weight was decreased at LD and MD and increased at the HD. Alterations in adrenal function may also underlie, in part, the observed effects upon fertility.

Increased developmental variations were seen in both rats and rabbits. In the data as presented, the skeletal system was affected, primarily at doses causing maternal toxicity.

Two developmental toxicology studies were conducted. The earlier study used insensitive detection methods yet still showed delays in eye opening and negative geotaxis. The second study did not present any data.

Genotoxicity assays included the Ames assay, in vivo mouse micronucleus test and in vitro cytogenetics using Chinese hamster ovary cells. An increase in aberrations per cell and the percent cells with aberrations was reported for one concentration (576µg/ml) +S9 activation given 10 hours of incubation. This effect was not seen in the 400 and 800 µg/ml concentrations incubated for 20 hours.

A radiolabeled tissue distribution study indicated that presence of drug-derived radioactivity in the retinal pigment epithelium of pigmented animals. The elimination half-life of radioactivity from the RPE after a single dose of drug was 23 days. While melanin binding is not unusual, three concerns are presented:

1) There is no indication in the study reports that a qualified veterinary ophthalmologist has examined any of the animals used in the toxicology studies.
2) There is no characterization of the effect of repeated dosing upon the pigmented structures of the eye or pigmented skin (e.g., is there a potential for phototoxicity?)
3) The mechanism of action of the drug is proposed to be an alteration of mitochondrial fatty acid metabolism. The highly metabolically active RPE is rich in mitochondria. If the drug truly alters (down-regulates) mitochondrial fatty acid oxidation, what effect will this have on the RPE?

The drug is highly metabolized with over 100 metabolites reported. Several of these (approximately 8) are present in multiple species, including humans, in significant amounts. Three study reports from July 2002 report the identification of CVT4786, a

major new metabolite in rats, mice and dogs. In humans, CVT4786 has been found in amounts up to 30-40% of the parent drug. No pharmacologic/toxicologic characterization of that major metabolite has been located in the submission. Overall, there is a lack of systematic characterization of the pharmacology/toxicology of the major metabolites.

B. Pharmacologic Activity: In anesthetized dogs, ranolazine caused an increase in coronary artery blood flow and a decrease in systolic arterial pressure.There is data to show that ranolazine may in fact decrease fatty acid oxidation. There is data to show that ranolazine also binds to a number of receptors which may in turn secondarily affect cardiac energy metabolism.

C. Nonclinical Safety Issues Relevant to Clinical Use: see above. The ability of the drug and metabolites to interact with cardiac ion channels creates the potential for effects upon cardiac repolarization. Based upon the cardiovascular safety study, there is a concern that use of this drug in patients with congestive heart failure could precipitate a marked decrease in contractility and left ventricular function and increased afterload (TPVR). The neurologic effects seen in animals create concern for potential neurologic effects in humans.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: unclear, possibly reducing cardiac oxygen demand at a given workload.

Mechanism of action: unclear. Possibly calcium channel blockade

Drug activity related to proposed indication: unclear. Possibly decreasing cardiac oxygen demand.

Secondary pharmacodynamics: cardiovascular deterioration (loss of contractility and increased afterload (TPVR)), central, multifocal neurologic effects and some effect on the HPA axis.

SpectrumScreen™ Pharmacology Report for Ranolazine free Base August 11, 1997. Panlabs 138249. The compound dissolved in 0.5% DMSO was tested in radioligand binding assays for maximum total binding and nonspecific binding against a number of different receptors. Nonspecific binding was defined as the proportion of total binding not displaced by unlabeled ligand. The summary of results meeting significance criteria is shown below.

It may be seen that the free base interacts with a number of receptors. Some interactions do not quite achieve significance but are close, such as Ca^{2+} channel binding.

Significant responses (meeting significance criteria) were noted in the assays listed below:

CAT.#	PRIMARY ASSAY NAME	CONC.	% INH.	IC_{50}	K_i	nH
20310	Adrenergic α_{1A}	10 µM	53			
20320	Adrenergic α_{1B}	10 µM	50			
20352	Adrenergic α_{2A}	10 µM	57			
20411	Adrenergic β_2	10 µM	58			
27111	Serotonin 5-HT_{1A}	10 µM	51			

CAT.#	SECONDARY ASSAY NAME	DOSE/CONC.	CRITERIA	RESULT	ACTIVITY
46501	Adrenergic α_1 Antagonism	10 µM	≥ 50%	61	Y
46900	Serotonin 5-HT_{1A} Agonist	10 µM	≥ 50%	91	Y

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EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138249
CODE: CV-10
E4-NE-002

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

CAT.#	TARGET	CONC.		PERCENT INHIBITION %	IC50	Ki	nH
20050	Adenosine A_1	10.0	µM	-5			
20060	Adenosine A_{2A}	10.0	µM	-7			
20070	Adenosine A_3	10.0	µM	17			
◆ 20310	Adrenergic α_{1A}	10.0	µM	57			
◆		10.0	µM	53			
		1.0	µM	22			
		0.1	µM	24			
		0.01	µM	4			
◆ 20320	Adrenergic α_{1B}	10.0	µM	50			
		10.0	µM	43			
		1.0	µM	10			
		0.1	µM	-4			
		0.01	µM	-1			
◆ 20362	Adrenergic α_{2A}	10.0	µM	59			
◆		10.0	µM	57			
		1.0	µM	14			
		0.1	µM	18			
		0.01	µM	4			
20370	Adrenergic α_{2B}	10.0	µM	36			
20401	Adrenergic β_1	10.0	µM	43			
◆ 20411	Adrenergic β_2	10.0	µM	65			
◆		10.0	µM	58			
		1.0	µM	11			
		0.1	µM	19			
		0.01	µM	-5			
20420	Adrenergic β_3	10.0	µM	31			
21000	Angiotensin AT_1	10.0	µM	-8			
21010	Angiotensin AT_2	10.0	µM	-14			
21150	Bombesin	10.0	µM	27			
21250	Bradykinin B_1	10.0	µM	6			
21260	Bradykinin B_2	10.0	µM	7			
21450	Ca^{2+} Ch. (L), Benzothiazepine	10.0	µM	23			
21460	Ca^{2+} Ch. (L), Dihydropyridine	10.0	µM	17			
21500	Ca^{2+} Ch. (L), Phenylalkylamine	10.0	µM	-17			
21600	Ca^{2+} Channel (N)	10.0	µM	-1			
21701	Cannabinoid CB_1	10.0	µM	-6			
21710	Cannabinoid CB_2	10.0	µM	-8			
21801	Cholecystokinin CCK_A	10.0	µM	21			
21811	Cholecystokinin CCK_B	10.0	µM	4			
21950	Dopamine D_1	10.0	µM	8			

Results with ≥50% stimulation or inhibition are **boldfaced**. (Negative values correspond to stimulation of binding or enzyme activity)

138249BP.CV SpectrumScreen

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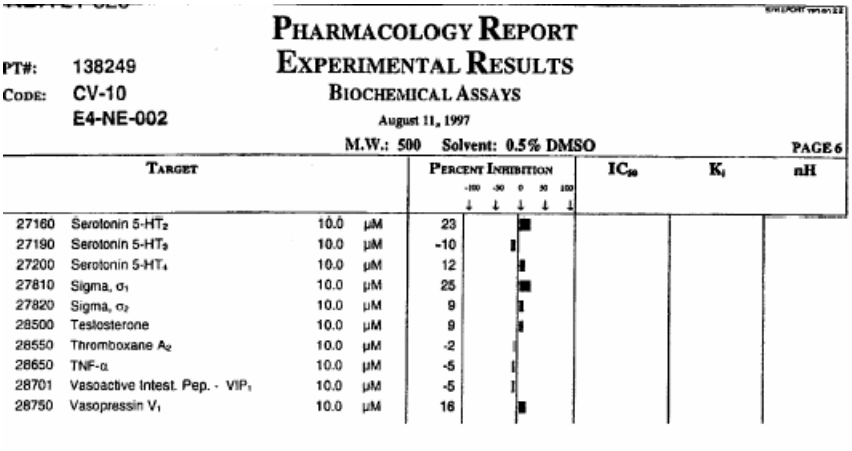
PT#: 138249
CODE: CV-10
E4-NE-002

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

	TARGET			PERCENT INHIBITION (-100 -50 0 50 100)	IC_{50}	K_i	nH
21960	Dopamine D_{2L}	10.0	µM	8			
21980	Dopamine D_3	10.0	µM	12			
21990	Dopamine $D_{4.2}$	10.0	µM	13			
22020	Dopamine D_5	10.0	µM	22			
22400	Endothelin ET_A	10.0	µM	-5			
22410	Endothelin ET_B	10.0	µM	1			
22600	Estrogen	10.0	µM	1			
22650	$GABA_A$, Agonist Site	10.0	µM	5			
22660	$GABA_A$, Bezodiazepine, Cen.	10.0	µM	16			
22680	$GABA_A$, Chloride Channel	10.0	µM	18			
23200	Glucocorticoid	10.0	µM	8			
23270	Glutamate, Kainate	10.0	µM	-3			
23280	Glutamate, NMDA, Agonist	10.0	µM	-7			
23900	Glycine, Strychnine-Sens.	10.0	µM	-3			
23950	Histamine H_1, Central	10.0	µM	16			
23970	Histamine H_2	10.0	µM	15			
23980	Histamine H_3	10.0	µM	14			
24350	Interleukin-1α	10.0	µM	-7			
25060	Leukotriene D_4	10.0	µM	5			
25260	Muscarinic M_1	10.0	µM	-17			
25270	Muscarinic M_2	10.0	µM	-3			
25280	Muscarinic M_3	10.0	µM	21			
25290	Muscarinic M_4	10.0	µM	-15			
25300	Muscarinic M_5	10.0	µM	-4			
25551	Neurokinin NK_1	10.0	µM	19			
25560	Neurokinin NK_2	10.0	µM	5			
25700	Neuropeptide Y_1	10.0	µM	-6			
25710	Neuropeptide Y_2	10.0	µM	31			
25860	Nicotinic Acetylcholine, Cen.	10.0	µM	5			
26010	Opiate-δ	10.0	µM	17			
26020	Opiate-κ	10.0	µM	-17			
26040	Opiate-µ	10.0	µM	17			
26560	K^+ Channel [K_{ATP}]	10.0	µM	6			
26570	K^+ Channel [K_V]	10.0	µM	7			
26580	K^+ Channel [SK_{Ca}]	10.0	µM	-4			
◆ 27111	Serotonin 5-HT_{1A}	10.0	µM	65			
◆		10.0	µM	51			
		1.0	µM	30			
		0.1	µM	23			
		0.01	µM	21			

Results with ≥50% stimulation or inhibition are **boldfaced**. (Negative values correspond to stimulation of binding or enzyme activity)



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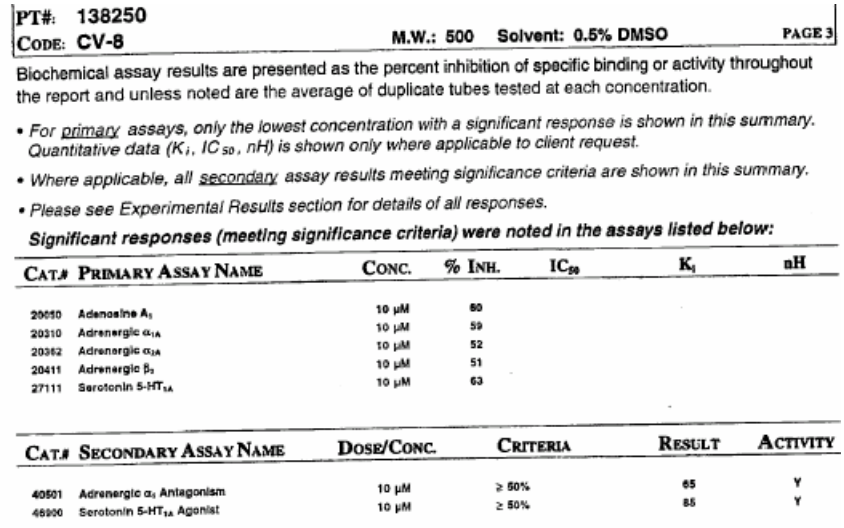
	TARGET			PERCENT INHIBITION	IC_{50}	K_i	nH
27160	Serotonin 5-HT_2	10.0	µM	23			
27190	Serotonin 5-HT_3	10.0	µM	-10			
27200	Serotonin 5-HT_4	10.0	µM	12			
27810	Sigma, σ_1	10.0	µM	25			
27820	Sigma, σ_2	10.0	µM	9			
28500	Testosterone	10.0	µM	9			
28550	Thromboxane A_2	10.0	µM	-2			
28650	TNF-α	10.0	µM	-5			
28701	Vasoactive Intest. Pep. - VIP_1	10.0	µM	-5			
28750	Vasopressin V_1	10.0	µM	16			

The screening lab came to the conclusion that displacement of radioligand from adrenergic α1A and adrenergic α1B binding sites was related to functional receptor antagonism. Displacement of radioligand from serotonin 5-HT1A binding sites appeared to be related to functional receptor agonism. Displacement of radioligand from adrenergic α2A and adrenergic β2 were concluded to be unrelated to functional receptor agonism or antagonism.

SpectrumScreenTM pharmacology report for the S-enantiomer of ranolazine free base. August 11, 1997. Panlabs138250

The S-enantiomer of ranolazine was dissolved in 0.5% DMSO and tested against the same panoply of receptors. The summary of significant responses is shown below.



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Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report and unless noted are the average of duplicate tubes tested at each concentration.

- *For primary assays, only the lowest concentration with a significant response is shown in this summary. Quantitative data (K_i, IC_{50}, nH) is shown only where applicable to client request.*
- *Where applicable, all secondary assay results meeting significance criteria are shown in this summary.*
- *Please see Experimental Results section for details of all responses.*

Significant responses (meeting significance criteria) were noted in the assays listed below:

CAT.#	PRIMARY ASSAY NAME	CONC.	% INH.	IC_{50}	K_i	nH
20650	Adenosine A_1	10 µM	60			
20310	Adrenergic α_{1A}	10 µM	59			
20362	Adrenergic α_{2A}	10 µM	52			
20411	Adrenergic β_2	10 µM	51			
27111	Serotonin 5-HT_{1A}	10 µM	63			

CAT.#	SECONDARY ASSAY NAME	DOSE/CONC.	CRITERIA	RESULT	ACTIVITY
40501	Adrenergic α_1 Antagonism	10 µM	≥ 50%	65	Y
46900	Serotonin 5-HT_{1A} Agonist	10 µM	≥ 50%	85	Y

The S-enantiomer, like the free base, showed activity for the adrenergic receptors as well as the calcium channel and opiate receptors. The sponsor also came to the same conclusion that displacement of radioligand from adrenergic α1A and adrenergic α1B binding sites was related to functional receptor antagonism. Displacement of radioligand from serotonin 5-HT1A binding

sites appeared to be related to functional receptor agonism. Displacement of radioligand from adrenergic α2A and adrenergic β2 were concluded to be unrelated to functional receptor agonism or antagonism.

SpectrumscreenTM pharmacology report for the R-enantiomer of ranolazine free base. August 11, 1997. Panlabs 138251.

The R-enantiomer was dissolved in 0.5% DMSO and tested against the various receptors as was done with the free base and S-enantiomers. The lab reported that no significant responses were observed in any primary assay. It may still be seen that there was substantial binding activity for the adrenergic receptors, serotonin 5HT1A, calcium channels and opiates.

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CAT.#	TARGET	CONC.		PERCENT INHIBITION %	IC50	Ki	nH
20050	Adenosine A_1	10.0	µM	-5			
20060	Adenosine A_{2A}	10.0	µM	8			
20070	Adenosine A_3	10.0	µM	-15			
20310	Adrenergic α_{1A}	10.0	µM	45			
20320	Adrenergic α_{1B}	10.0	µM	15			
20362	Adrenergic α_{2A}	10.0	µM	35			
20370	Adrenergic α_{2B}	10.0	µM	36			
20401	Adrenergic β_1	10.0	µM	-1			
20411	Adrenergic β_2	10.0	µM	11			
20420	Adrenergic β_3	10.0	µM	10			
21000	Angiotensin AT_1	10.0	µM	11			
21010	Angiotensin AT_2	10.0	µM	14			
21150	Bombesin	10.0	µM	-8			
21250	Bradykinin B_1	10.0	µM	15			
21260	Bradykinin B_2	10.0	µM	6			
21450	Ca^{2+} Ch. (L), Benzothiazepine	10.0	µM	18			
21460	Ca^{2+} Ch. (L), Dihydropyridine	10.0	µM	15			
21500	Ca^{2+} Ch. (L), Phenylalkylamine	10.0	µM	-16			
21600	Ca^{2+} Channel (N)	10.0	µM	5			
21701	Cannabinoid CB_1	10.0	µM	11			
21710	Cannabinoid CB_2	10.0	µM	15			
21801	Cholecystokinin CCK_A	10.0	µM	28			
21811	Cholecystokinin CCK_B	10.0	µM	23			
21950	Dopamine D_1	10.0	µM	2			
21960	Dopamine D_{2L}	10.0	µM	23			
21980	Dopamine D_3	10.0	µM	1			
21990	Dopamine $D_{4.2}$	10.0	µM	-2			
22020	Dopamine D_5	10.0	µM	19			
22400	Endothelin ET_A	10.0	µM	-7			
22410	Endothelin ET_B	10.0	µM	-3			
22600	Estrogen	10.0	µM	8			
22650	$GABA_A$, Agonist Site	10.0	µM	-13			
22660	$GABA_A$, Bezodiazepine, Cen.	10.0	µM	6			
22680	$GABA_A$, Chloride Channel	10.0	µM	5			
23200	Glucocorticoid	10.0	µM	9			
23270	Glutamate, Kainate	10.0	µM	12			
23280	Glutamate, NMDA, Agonist	10.0	µM	-15			
23900	Glycine, Strychnine-Sens.	10.0	µM	20			
23950	Histamine H_1, Central	10.0	µM	12			
23970	Histamine H_2	10.0	µM	20			

Results with ≥50% stimulation or inhibition are **boldfaced**. (Negative values correspond to stimulation of binding or enzyme activity)

138251BP.CV SpectrumScreen

NDA 21-526

PHARMACOLOGY REPORT
EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138251
CODE: CV-9
PA-18877-70

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

	TARGET			PERCENT INHIBITION	IC$_{50}$	K_i	nH
23980	Histamine H_3	10.0	µM	12			
24350	Interleukin-1α	10.0	µM	2			
25060	Leukotriene D_4	10.0	µM	7			
25260	Muscarinic M_1	10.0	µM	18			
25270	Muscarinic M_2	10.0	µM	16			
25280	Muscarinic M_3	10.0	µM	24			
25290	Muscarinic M_4	10.0	µM	13			
25300	Muscarinic M_5	10.0	µM	-4			
25551	Neurokinin NK_1	10.0	µM	-12			
25560	Neurokinin NK_2	10.0	µM	3			
25700	Neuropeptide Y_1	10.0	µM	-7			
25710	Neuropeptide Y_2	10.0	µM	4			
25860	Nicotinic Acetylcholine, Cen.	10.0	µM	21			
26010	Opiate-δ	10.0	µM	20			
26020	Opiate-κ	10.0	µM	-11			
26040	Opiate-µ	10.0	µM	4			
26560	K^+ Channel [K_{ATP}]	10.0	µM	10			
26570	K^+ Channel [K_V]	10.0	µM	6			
26580	K^+ Channel [SK_{Ca}]	10.0	µM	-4			
27111	Serotonin 5-HT_{1A}	10.0	µM	7			
27160	Serotonin 5-HT_2	10.0	µM	21			
27190	Serotonin 5-HT_3	10.0	µM	-11			
27200	Serotonin 5-HT_4	10.0	µM	17			
27810	Sigma, σ_1	10.0	µM	33			
27820	Sigma, σ_2	10.0	µM	33			
28500	Testosterone	10.0	µM	5			
28550	Thromboxane A_2	10.0	µM	7			
28650	TNF-α	10.0	µM	0			
28701	Vasoactive Intest. Pep. - VIP_1	10.0	µM	-3			
28750	Vasopressin V_1	10.0	µM	10			

Results with ≥50% stimulation or inhibition are **boldfaced.** (Negative values correspond to stimulation of binding or enzyme activity)

AT4927 The effect of RS-43285-193 on opioid receptor binding in Guinea Pig brain. November 1988. RS-43285-193 was tested for the ability to compete for mu, kappa and delta opioid receptor binding in guinea pig brain homogenates. Concentrations used were 0.1 nM - 100µM. Tritiated etorphine (kappa binding ligand), penicillamine-enkephalin (delta binding) and glyol (mu binding) were used as competitors. The opioid antagonist naloxone was used at several concentrations that included 15 nM, 21 nM and 146 nM.

Ranolazine was reported to be inactive at all concentrations tested for delta binding but showed activity at concentrations ≥ 10µM for mu and kappa binding. The only data presented was the textual comment that at 100µM, ranolazine caused 27% and 24% inhibition of mu and kappa receptor binding respectively. Naloxone was reported to give 50% inhibtion in mu, kappa and delta binding assays at 15nM, 21nM and 146 nM respectively. By comparison, ranolazine showed lower affinity for the opioid receptors.

AT4707 The effects of RS-43285-197 in K^+ depolarised smooth muscle; interactions with Ca^{2+} and Ca^{2+} channel activators. January 1986.

Taenia were isolated/prepared from the cecums of female guinea pigs and established in organ baths with a calcium free K^+-Tyrode solution. Cumulative calcium response curves for Ca^{2+} (0.01, 0.03, 0.1, 0.3, 1,3 mmol/l) were obtained by increasing calcium at 3 minute intervals. In some experiments, preparations were incubated with RS-43285 for 30 minutes prior to being half-maximally contracted with Ca^{2+} (0.3×10^{-3} mol.l^{-1}). After the contractions had stabilized, a concentration-response curve was obtained to the Ca^{2+}channel activators Bay K 8644 (10^{-9} to 3×10^{-6} mol.l^{-1}) or palmitoyl carnitine (10^{-5} to 3×10^{-4} mol.l^{-1}).

Results: Concentrations of RS-43285 from 10^{-5} to 10^{-3} mol.l^{-1} antagonized responses to Ca^{2+} in the K^+ depolarized taenia preparations. The sponsor reported that the combination of ranolazine with either Bay K 8644 or palmitoyl carnitine did not affect the augmentation of calcium-induced contractions by the latter two compounds.

The sponsor concluded that although ranolazine's inhibition of K+-depolarized smooth muscle was indicative of calcium channel blockade, "the concentration of RS-43285 necessary to achieve this effect is so high,… that RS-43285 must act as an anti-anginal agent by some other mechanism. This suggestion is further confirmed by the lack of effect with the Ca2+ channel activators Bay K 8644 and palmitoyl carnitine."

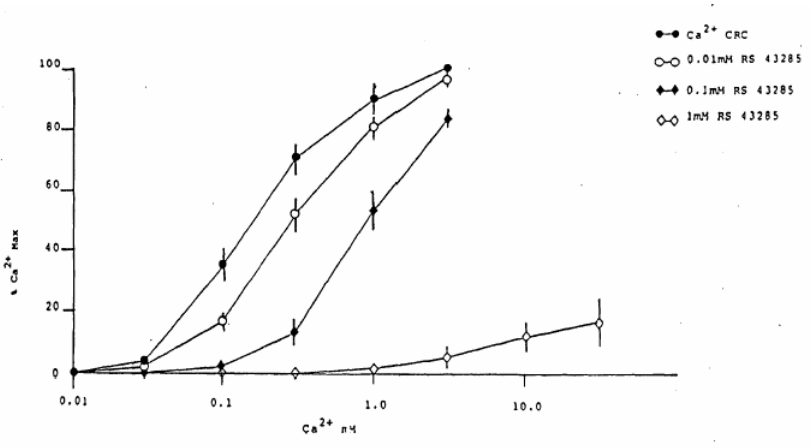


Figure 1: The effect of RS-43285 on K^+-depolarised guinea-pig taenia smooth muscle preparations.

AT5425 Effects of RS-43285-193 on guinea pig cardiac ventricular action potentials in vitro under normal and ischemic conditions. Dec. 1990

Female Duncan-Hartley guinea pigs were euthanized and the hearts collected in ice cold physiologic "salt" solution. Free running papillary muscles, 1 mm diameter were dissected from the right ventricle and transferred to a perfusion chamber. The muscle preparations were perfused with a physiologic salt solution (PSS). Solutions were preheated and oxygenated. The preparation was stimulated at a basal frequency of 1 Hz and supramaximal intensities. Resting membrane potentials and action potentials were recorded from an intracellular microelectrode. The effects of each concentration of ranolazine were monitored for over 30 minutes. At the end of each 30 minute period the stimulation frequency was increased to 3.3 Hz to investigate use-dependent blockade of sodium channels.

In a separate set of tissues, the effects of ranolazine on ischemia-induced changes in electrical and mechanical activity were examined. After electrode placement, the tissue was exposed to physiologic salt solution in which the glucose had been replaced by mannitol and which was vigorously gassed with N_2. Changes in APD, contractility and tension were monitored every 5 minutes for 30 minutes. The tissue was then superfused with standard physiologic salt solution for 40 minutes. Drug or vehicle was then added to the superfusing solution and equilibration allowed for 20 minutes. In the continuing presence of drug or vehicle the tissue was then exposed to glucose-free PSS(gassed with N2) for a further 30 minute period.

Results:

Over the concentration range of 0.1, 1, 10 and 30 μM, ranolazine had no effect on maximum diastolic potential. At concentrations of 10^{-6}, 10^{-5} and $3x10^{-5}$, a dose-dependent prolongation of action potential duration was apparent. The maximum rate of depolarisation at both 1.0 and 3.3 Hz was decreased compared to control at these concentrations. However, the only significant change was at the highest concentration at a frequency of 3.3 Hz. The sponsor's results are shown below. Data are mean ±sem from 4 separate experiments.

Effects of RS-43285-193 on maximum diastolic (resting) potential, maximum rate of depolarisation (V_{max}) and action potential duration

Concentration (M)	Membrane Potential (mV)	Maximum rate of Depolarisation 1.0 Hz (V/s)	3.3 Hz	Action Potential Duration (ms)
0	-82.4 ± 2.46	209.3 ± 15.00	202.9 ± 13.76	194.0 ± 2.37
10^{-7}	-83.8 ± 1.51	202.5 ± 8.82	195.4 ± 8.77	193.0 ± 4.08
10^{-6}	-82.7 ± 1.70	203.6 ± 9.28	194.3 ± 7.66	195.1 ± 2.77
10^{-5}	-82.8 ± 2.27	200.4 ± 8.50	171.1 ± 6.44	211.8 ± 2.06**
$3x10^{-5}$	-83.5 ± 2.05	191.8 ± 6.57	134.3 ± 8.76**	224.4 ± 4.11**

** indicates a value significantly different from control ($p < 0.01$, Analysis of Variance and Dunnett's t-test).

'e mear s.e. of mean from 4 separate experiments.

Ischemia-induced increases in tension were larger in the presence of drug than in control tissues . The sponsor conludes that the increase in APD was either due to calcium or potassium channel effects. The conclusion goes on to state that as ranolazine has no positive inotropic effects at the concentrations used, the effect is probably due to blockade of cardiac potassium channels. Given the effects in this study on Vmax and APD, the sponsor summarizes that in this model system, the drug has effects similar to Class I and Class III anti-arrythmic drugs. The study would be stronger for the inclusion of positive controls, either historical or concurrent.

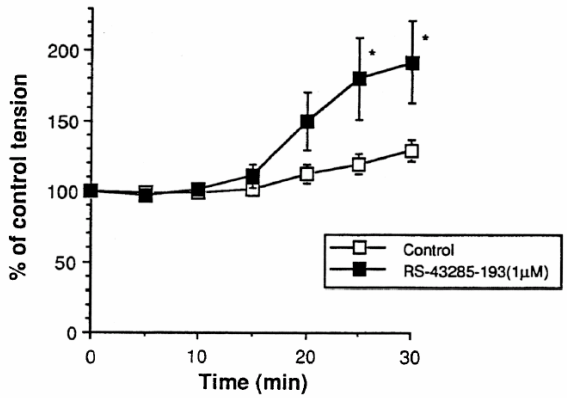


Figure 6.
Effects of RS-43285-193 on ischaemia-induced changes in tension.

Data are means ± s.e. mean (n=4-5 preparations). * indicates a significant difference relative to vehicle control tissues ($p<0.05$; Analysis of Variance and Bonferroni modified t-test).

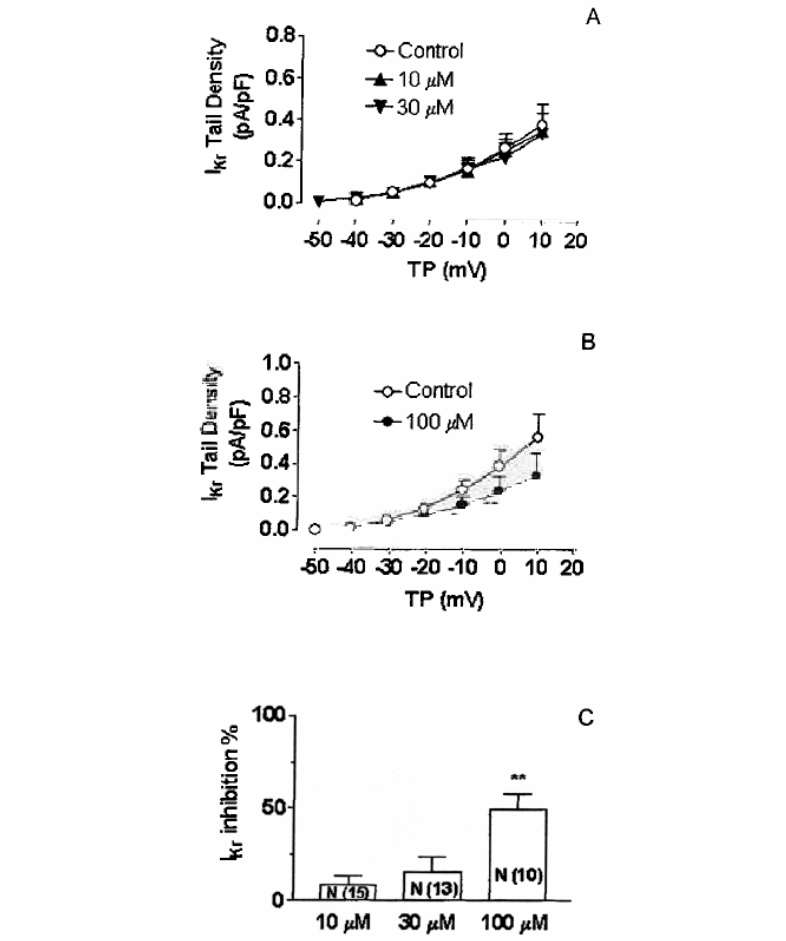
CVT303.041P Effects of ranolazine on I_k , I_{Na} and I_{Ca} in canine atrial myocytes. September 2002.

Adult mongrels were anesthetized and the hearts collected and immersed in Tyrode solution. Left atrial myocytes were isolated via collagenase perfusion and kept in high K+ storage. Nimodipine (1μM), dofetilide(1μM) and atropine(1μM) were used to block I_{Ca}, I_{kr} and acetylcholine-dependent K+ current respectively. General voltage clamp techniques were used.

Total I_k currents were elicited by 3-s depolarizing pulses from –50 to +70 mV followed by 2-s repolarizations to –40 mV to observe tail currents. I_{ks} was studied in the presence of E-4301 (5μM) to inhibit I_{kr}. I_{kr} recordings were obtained with 200 ms pulses from –60 mV to +10mV followed by 2 s repolarizations to –40 mV to observe tail currents.

I_{Ca} was recorded on 240 ms depolarizing pulses from –50mV to voltages ranging from –40mV to +60 mV.

Figure 2 Effect of Ranolazine on I_{Kr} Based on Tail Current Measurements



Panel A: Effect of 10 and 30 μM ranolazine on I_{Kr} tail currents (in pA/pF). Panel B: Effect of 100 μM ranolazine on I_{Kr} tail currents (in pA/pF). *P<.05; **P<.01 vs control. Panel C: Percent reduction in I_{Kr} by ranolazine concentrations indicated, based on tail currents following an activating step to 0 mV.

100% inhibition of the calcium channels was achieved.

I_{Na} was recorded during 40 ms depolarizations applied at 1 and 2 Hz from a holding potential of –140 to –40 mV.

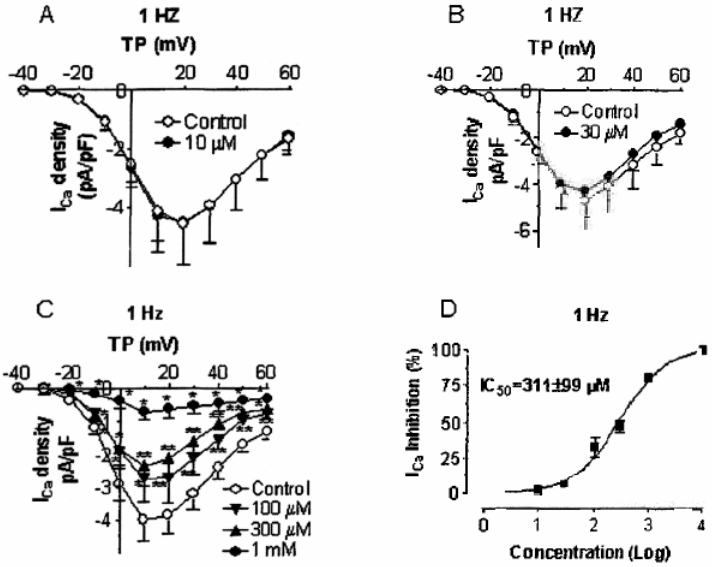
Recordings were performed before drug appliction as the control, after 10 minutes of superfusion with ranolazine and after washout (time undefined) of drug.

Results:
Note: The numbers given below were taken from the text of the results section.
Ranolazine caused a dose-dependent decrease in I_{kr} tail density: 8.2%(10μM), 15.2%(30μM) and 49.3%(100μM) compared to control conditions.

Ranolazine decreased I_{ks} density by 16%(100μM), 17% (300μM) and 27%(1000μM).

Ranolazine also had an affect on I_{Ca} at concentrations ≥100 μM. Based upon the graphical results,

Figure 4 Inhibition of I_{Ca} by Ranolazine During Depolarizing Pulses at a Frequency of 1 Hz



Panels A, B, and C: Current density-voltage relations for I_{Ca} under control conditions and in the presence of 10 (n = 6), 30 (n = 6), 100 (n = 8), 300 (n = 7), and 1000 μM (n = 5) ranolazine. Panel D: Concentration-response curve for ranolazine inhibition of I_{Ca} (at +10 mV, n = 8). Results are mean ± SEM. *P < 0.05 and **P < 0.01 vs control.

In canine atrial myocytes under the conditions used in this study, ranolazine inhibits I_{kr}, I_{ks} and I_{Ca} with a potency order of $I_{Kr}>I_{Ca}>I_{Ks}$ but an efficacy order of $I_{Ca}>I_{Kr}>I_{ks}$. The study would be stronger for the inclusion of positive controls or comparator compounds.

CVT303.048-P Electrophysiological effects of ranolazine in the guinea pig heart in vivo. March 2002.

The report describes this as a novel in vivo guinea pig model. Anesthetized guinea pigs were intubated and ventilated with room air. The right carotid artery was cannulated for direct blood pressure measurements. A custom made catheter was introduced through the left carotid artery into the left ventricle to record endocardial monophasic action potentials (MAP). Another electrode catheter was positioned in the right atrium and ventricle for atrial and ventricular pacing.

The treatment groups included:

Group I	Control
Group II	Vehicle control
Group III	Ran 2.5 mg/kg bolus + 90µg/kg/min iv (LD Ran)
Group IV	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv
Group V	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv + Ran 2.5 mg/kg bolus + 90µg/kg/min iv
Group VI	Ran 5 mg/kg bolus + 180 µg/kg/min, iv (HD Ran)
Group VII	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv + HD Ran

For all groups, electrophysiological parameters were recorded and determined following instrumentation, a 20 minute stabilization period and a 15 minute saline infusion, vehicle infusion,quinidine, low and high doses of Ranolazine and in the presence of quinidine. Blood samples for plasma level determination of drug were collected at 15 minutes following the administration of Ran.

Results: We do not know the stability of this model over the duration of time that it took to collect the data. Ranolazine caused a dose-related increase in $MAPD_{100}$. The effect was additive with quinidine. The sponsor's results are shown below.

Table 1: Sinus cycle length (SCL), systemic blood pressure (BP), and left ventricular monophasic action potential duration at 100% repolarization ($vMAPD_{100}$) before (Baseline) and 15 min after commencement of various treatments (Treatment).

Group	Baseline			Treatment (15 min)		
	SCL (ms)	BP (mm Hg)	$vMAPD_{100}$ (ms)	SCL (ms)	BP (mm Hg)	$vMAPD_{100}$ (ms)
Control	241±8	51±2	123±9	248±11	51±3	123±9
Carrier	232±7	49±2	129±2	246±14	49±4	126±3
Quinidine	220±7	53±2	120±3	248±8*	54±3	134±5*
Low-dose Ranolazine	256±20	58±3	121±2	278±12	56±3	133±4
Low-dose Ranolazine + Quinidine	221±16	56±2	128±4	292±8*	54±2	152±6*
High-dose Ranolazine	215±6	49±5	118±4	260±10*	50±4	144±5*
High-dose Ranolazine + Quinidine	218±6	46±2	119±4	306±8*	46±3	166±5*

Table 2: Values of electrophysiological parameters measured before (control) and after treatment with Ranolazine (RAN), alone and in combination with Quinidine (Q). Vehicle (carrier), low dose RAN (2.5 mg/kg + 90 µg/kg/min, IV) and high-dose RAN (5 mg/kg + 180 µg/kg/min, IV), Quinidine (10 mg/kg + 50 µg/kg/min, IV), alone and in the presence of low and high doses of RAN.
Values are mean ± SEM.

Parameter (msec)	Control (n=8)	Carrier (n=8)	Low RAN (n=8)	Q (n=8)	Low RAN + Q (n=8)	High RAN (n=8)	High RAN + Q (n=8)
		After 15 min infusion					
VRP_{90}	96±2	95±2	107±4	107±3	124±2*	127±5†	143±5††
VRP_{80}	94±3	92±2	106±4	106±3	120±3*	124±5†	136±6††
ARP_{90}	67±3	67±4	75±2	82±4	91±2*	93±6†	113±8††
ARP_{80}	67±3	66±4	76±3	81±4	90±2*	92±6†	111±8††
$SNRT_{90}$	290±18	266±10	336±24*	253±6	284±10	325±13*	326±13*
$SNRT_{80}$	296±18	269±13	348±29*	265±7	293±8	326±16*	335±15*
AVB_W	151±4	148±6	165±12	144±3	165±11	162±6	189±10*

$VRP_{90,80}$, $ARP_{90,80}$ and $SNRT_{90,80}$ are the ventricular and atrial refractory periods and sinus node recovery times, respectively, measured at 80% and 90% of SCL. AVB_W is the AV nodal Wenckebach point defined as the longest atrial cycle length at which second-degree AV block occurs. * P<0.05 vs. control, carrier, Quinidine alone; † P<0.05 vs. control, carrier, L_RAN; †† vs. all other groups.

ECGs were monitored but ECG intervals including QT, QRS, PR etc were not provided.

From the sponsor's data above it may be seen that repolarization periods of both the atria and ventricles increased with increasing dose of ranolazine. Both doses of ranolazine when combined with quinidine caused a greater effect than either drug alone.

CVT303.012N: Effects of ranolazine on membrane potentials and currents of guinea pig isolated ventricular myocytes .January 2002.
Single ventricular myocytes were isolated from the hearts of adult male guinea pigs (breed unspecified). Myocytes were placed in a recording chamber and superfused with Tyrode solution at 35°C. Transmembrane voltages were measured with glass electrodes containing appropriate solutions. Initial experiments showed that ranolazine increased the action potential duration and $I_{Ca(L)}$.Therefore additional studies were conducted with nitrendipine for comparison.
The effect of ranolazine on membrane potentials: action potentials were induced by 5 ms-depolarizing pulses applied at a frequency of 0.5 Hz. The duration of the action potential was measured at APD_{50} and APD_{90}. Ranolazine was applied at concentrations of 1,3,10, 30 and 100 µmol/l. The effect at each concentration was determined on 5 myocytes.

Resting membrane potential (RMP) was determined as the stable diastolic potential. The effect of ranolazine on the RMP was determined after the cells were treated with 10, 30 or 100 µmol/l ranolazine.

Effect on I_{k1}: Ik1 was elicited by a 4.8 second ramp voltage clamp pulse from –130-+50 mV at a frequency of 0.16 Hz. The amplitude of the current of 4 myocytes ± ranolazine (1-30 μmol/l) was determined.

Effect on $I_{Ca(L)}$: A 150-200 ms voltage clamp pulse from –40mV to 0mV was applied at a frequency of 0.5Hz and K+ in both Tyrode and pipette solutions was replaced by equimolar Cs+. The amplitude of the $I_{Ca(L)}$ was determined as the maximal inward current. Ranolazine was applied at concentrations of 3 and 30 μmol/l. Each concentration was tested on 5-6 myocytes.

Effect on I_k: IK was elicited by a 1-s depolarizing pulse from –40mV to +30mV at a frequency of 0.16 Hz. The amplitude of the tail current was measured to determine the amplitude of Ik. 30μmol/l Ran was tested on 6 myocytes.

Nitrendipine: The inhibitory effects of nitrendipine on $I_{Ca(L)}$ at concentrations of 0.1-1 μmol/l were tested. It was found that nitrendipine at 0.1 μmol/l mimicked the effect of ranolazine on $I_{Ca(L)}$. Further experiments were therefore performed to assess the affect of this concentration of nitrendipine on the APD.

Results: The sponsor's results are shown below. Ranolazine at concentrations of 1-100μM shortened APD. When expressed as a percentage of the control: 7±2 %(1 μM,APD_{50}), 18±2% (100 μm, APD_{50}) and 5±2%(1 μM, APD_{90}), 12±1% (100 μM, APD_{90}).
No effect on Ik1 was shown.
Ranolazine caused a decreased amplitude of $I_{Ca(L)}$ which was only partly reversible after washout of the drug. Nitrendipine at 0.1 μmol/l mimicked the effect of ranolazine on the calcium channel.
Reviewer's Summary of Results

	control	Ranolazine (μM)				
		1	3	10	30	100
APD_{50}(ms)	238± 20(5)	222± 20*		208±15		
	246± 17(5)		221± 15*		206± 14*	
	249± 23(5)					203± 15*
APD_{90}(ms)	272± 21(5)	257± 18		246± 15*		
	279± 18 (5)		254± 15*		248± 16*	
	284± 24 (5)					249± 18*
RMP (mV)	-80.9±1.3(14)				-80.8± 1.3	
$I_{Ca(L)}$(nA)	1.30±0.25 (5)		1.21± 0.24*			
	1.13±0.25 (6)				0.970.20*	
$I_{k(tail)}$ (pA)	155± 16 (6)				107±28*	

The sponsor was specifically asked (February 25, 2003) to identify the studies that were crucial to support the proposed mechanism of action and electrophysiological claims. The following are summary reviews of those studies.

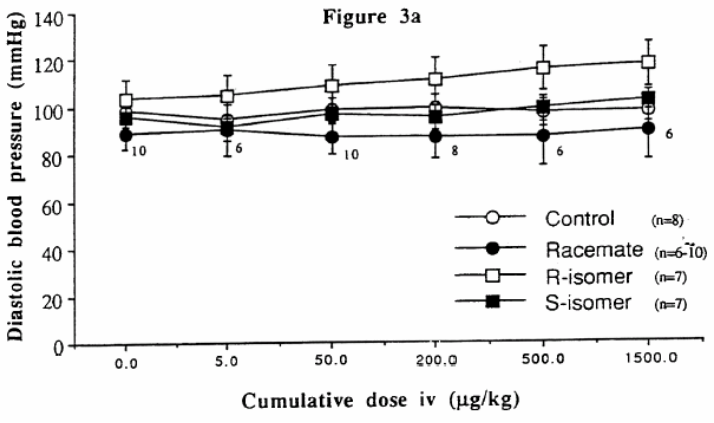
Mechanism of Action

AT6055:The effect of ranolazine enantiomers, RS-43285-197 (s-isomer) and RS-43285-198 (r-isomer), compared to ranolazine racemate (RS-43285-193) on ischemic ECG changes and hemodynamic function in the dog model of transient myocardial ischemia. August 1988

Adult Beagles of both sexes were anesthetized and subjected to cardiac pacing at 50-80 beats per minute above resting heart rate for 1 minute prior to LAD occlusion. Pacing plus occlusion was carried out for another 2 minutes. Ten minutes of rest was followed by another pacing event. Episodes of pacing and occlusion were repeated at least four times or until 2 consecutive challenges produced the same degree of S-T segment elevation with a return to baseline between challenges. ST segment measurements were made at 45, 60, 75, 90 and 105 seconds into the occlusion period and at 2 second intervals (10 values) after termination of occlusion and pacing. Two pre-drug conditioning S-T segment elevations for each electrode were averaged and this value taken as 100%. Each test event thereafter was expressed as a percentage of this baseline value. The dose range covered cumulative iv doses of 5, 15-20, 50, 200, 500 and 1500 μg/kg with each successive dose increment being injected 5 minutes into the reperfusion phase following each ischemic challenge. The treatment group sizes were: control (n=9), racemate (n=13), R isomer (n=7), S isomer (n=8).

Results: Repeated ischemic episodes produced S-T elevation. The time-related rise after injury was described as small compared to that apparent upon reperfusion. Some 77% of the dogs (n=35) showed a range of mean ±se percentage increase of S-T elevation of 180%-400% (259±10%, n=27). There was a wide range of baseline S-T segment elevation in response to ischemia. Results were presented as percentage change of the baseline. The use of percentages makes one wonder what effect would have been observed had the ranges of absolute response been shown. Note also that the acute nature of this model means that there is no time for electrical remodeling or alterations to the myocardium. This is essentially a healthy heart and conduction system. Blood pressure changes were shown graphically, with minor changes apparent but unquantifiable given the presentation. There appeared to be a dose-related increase in diastolic blood pressure with the R-isomer. Heart rate changes were apparent in the graphical presentations. Based on the results presented, the enantiomers and the racemate appear to decrease ST segment elevation as a percentage of baseline in this model.

Figure 3a



The effect of ranolazine racemate and its R- and S- isomers on S-T segment changes in epicardial electrocardiograms from the dog ischaemic myocardium stressed by electrical pacing

Insult phase

Dose mcg/kg	Untreated (n=9)	S-isomer (n=8)	R-isomer (n=7)	Racemate (n=6-13)
Control	100%	100%	100%	100%
5	101.0 ± 2.6	91.7 ± 5.1	98.3 ± 4.8	87.8 ± 3.8(7)**
20	103.8 ± 3.2	87.8 ± 3.3***	89.8 ± 2.7***	89.2 ± 2.4(6)***
50	102.1 ± 3.5	90.5 ± 8.1	89.1 ± 4.1*	76.6 ± 5.6(12)**
200	95.8 ± 3.0	82.5 ± 4.0**	87.9 ± 2.5+	70.8 ± 5.4(13)****
500	95.7 ± 2.8	76.5 ± 5.7***	77.4 ± 4.4***	75.0 ± 7.6(8)*
1500	93.5 ± 2.1	73.1 ± 4.2****	73.9 ± 2.8****	67.3 ± 6.5(8)***

Recovery phase

Dose mcg/kg	Untreated (n=8-9)	S-isomer (n=8)	R-isomer (n=7)	Racemate (n=7-11)
Control	100%	100%	100%	100%
5	101.4 ± 3.1	87.4 ± 4.4**	102.2 ± 7.2	98.3 ± 5.6(7)
20	99.5 ± 3.5	84.9 ± 4.3**	88.3 ± 4.3	90.2 ± 8.4(7)
50	102.8 ± 4.4	84.9 ± 5.9*	93.5 ± 5.4	87.3 ± 4.6(9)*
200	98.2 ± 2.7	82.6 ± 5.1**	89.0 ± 2.7	80.9 ± 3.9(11)****
500	98.4 ± 2.9	79.6 ± 5.1***	82.7 ± 4.1	81.1 ± 5.4(7)**
1500	98.4 ± 4.1(8)	75.1 ± 4.3***	74.9 ± 4.1***	76.7 ± 6.6(7)**

Data are means ± s.e.m. Numbers in parenthesis denote number of animals.
Statistical significances are shown: *P<0.05, **P<0.02, ***P<0.01, ****P<0.001 versus the corresponding untreated value by the Student's t-test.
Corresponding Bonferonni adjustment for multiple comparisons: *P<0.075(NS), **p<0.03, ***p<0.015, ****p<0.0015.
+p<0.03 for the effect of R-isomer versus Racemate; no other isomer to racemate or isomer to isomer comparisons were found to be statistically significantly different from one another.

AT3378: The effects of RS-43285 on the biochemical consequences of transient myocardial ischemia in the dog.

Three anesthetized dogs were paced at 50 – 80 bpm above resting heart rate for 1 minute before occlusion of the LAD. Pacing and occlusion lasted for 2 minutes during which time blood was collected from the coronary vein. The next cycle was begun after a 10 minute rest period. Five control challenges were performed followed by 3 post-drug challenges. RS-43285 was given iv for cumulative doses of 50, 200 and 500 μg/kg. Blood gases, electrolytes, glucose, lactate and ffa were measured.

"Production" and "uptake" parameters were calculated based on arterio-venous differences:

(arterio-coronary venous difference)/arterial x 100%

"consumption" = (arterio-venous difference)x regional myocardial blood flow ($ml.100g^{-1}.min^{-1}$) divided by (weight myocardial "ischemic bed"(g) x 100)

It was said that limitations of coronary blood flow data made calculation of the 500 μg/kg dose level impossible.

Results:
There was no table of values. Data was given graphically. Numbers were found in the textual description of results. The ischemic insult produced a 24% increase in FFA uptake from myocardial venous blood. Ranolazine caused a decrease in FFA uptake in the myocardium. At all 3 doses RS-43285 caused an increase in the disappearance of NEFAs from femoral arterial blood. The ischemic insult caused an increase in glucose extraction from 4.3% to 21.5%. Ranolazine decreased myocardial glucose extraction approximately 59% at all doses. The ischemic insult produced an 8% rise in myocardial glucose consumption. This was reduced to 2.9% and 4.6% by 50 and 200 μg/kg RS-43285 respectively (47%-66% fall from the basal value). Myocardial glucose consumption was also slightly decreased by 2.9% and 4.6% at 50 and 200 μg/kg respectively. It was stated that ischemia caused a decrease in oxygen consumption and that ranolazine further decreased the O_2 consumption. There was insufficient information to quantify this difference.

Between untreated controls and all drug-treated groups there was a decrease in myocardial venous lactic acid production expressed as % of baseline. There was no dose-response apparent. There were no differences between treated and untreated groups in cephalic venous or femoral arterial blood. K+ efflux during electrical stress pacing was decreased in blood samples collected from the 3 different blood vessels in treated animals compared to controls. NEFA uptake was decreased in all treated myocardial samples. NEFA uptake in cephalic venous samples were decreased at LD and increased at MD and HD compared to control. The percent glucose extraction decreased relative to control in all treated samples. Myocardial glucose consumption increased in control and treated groups. The increase in control was ~9% compared

to 3 and 5% in the treated groups. Myocardial O_2 extraction expressed as a percent increase from baseline was the same in control and LD (~5.5%), MD was at 3% and the HD was at 4.5%. It's not clear if these differences are variability of the system or real. There was no effect on myocardial pH values. Some of the differences between the groups, shown as percentage of baseline values, are very small (e.g. half a percentage) and one may wonder if a number of the differences are real.Use of a comparator compound might increase confidence that the model can be used to detect treatment differences.

CVT303.024-P Effects of acute intravenous ranolazine on left ventricular function in dogs with chronic heart failure.Oct. 1998-Aug 1999. Reported Nov 11, 1999

LV dysfunction and failure (LVEF 27±2%) was produced in 7 dogs by multiple sequential intracoronary microembolizations over a period of 5-15 weeks. Following a 2-3 week recovery period, hemodynamic and angiographic measurements were made before and 40 minutes after iv administration of ranolazine (0.5 mg/kg bolus and a 40 minute continuous iv infusion of 1.0 mg/kg/hour). Each animal served as its own control.

Results: There were no differences in arterial-coronary sinus samples for glucose, lactate or ffa after ranolazine. There were no significant effects on HR, systolic pressure, diastolic pressure, mean aortic pressure, LVEDP, peak dP/dt or several other parameters. There were non-significant increases in systolic aortic pressure, diastolic aortic pressure, mean aortic pressure, peak dP/dt and peak – dP/dt. Ejection fraction (%) increased from 27±5 to 35±5 ($p<0.001$) and stroke volume (SV, ml) went from 20±4 to 27±4 ml ($p<0.001$). The author of the report offered the opinion that the assays were insufficiently sensitive to show differences in the measured biochemical parameters.

CVT303.035-P Effects of acute intravenous ranolazine in cardiac function and mechanical efficiency in dogs with heart failure. September, 2000.

Eight anesthetized, healthy dogs were measured before and after dobutamine administration for LVEF, SV, coronary blood, MVO2 and the arterial-coronary sinus difference for glucose, lactate and ffa. Subsequent LV dysfunction and heart failure was produced by multiple sequential intracoronary microembolizations. Measurements were then repeated ± ranolazine or dobutamine.

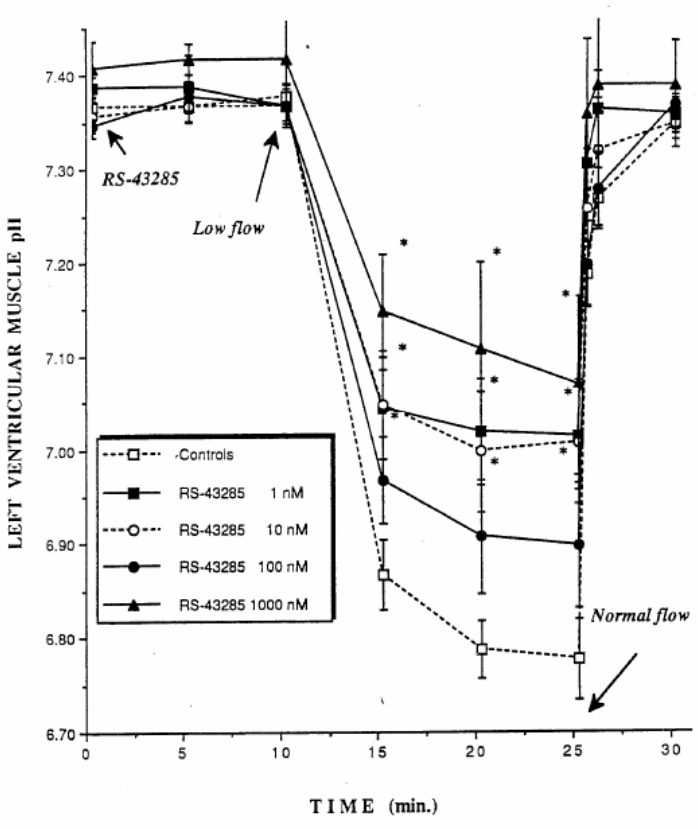
Results: In dogs with heart failure, dobutamine caused an increase in FFA uptake compared to the pretreatment levels ($p<0.01$). There was also a significant decrease in arterial glucose concentration ($p<0.02$). Ranolazine had very little effect on cardiac function in healthy dogs. It did increase glucose and ffa uptake in healthy dogs and decreased ffa and glucose uptake in heart failure dogs. There was no increase in MVO2 with ranolazine treatment although the LV efficiency increased.

AT4537: Anti-ischemic effects of ranolazine (RS-43285) in isolated rat hearts subjected to low perfusion flow. November 1988

Hearts were collected from male Sprague-Dawley rats then perfused with Kreb's solution. A microelectrode was implanted into the ventricular wall. Hearts were perfused at 14 ml/min to obtain a stable baseline ventricular pH. By adjusting the pump speed the flow was decreased to 1 ml/min for 15 minutes. Flow was restored to the initial rate for 15 minutes. Measurements of coronary flow were made at 30 seconds, 1 minute and 5 minutes and 5 minute intervals thereafter. Infusions of ranolazine (1 nM, 10 nM, 100 nM and 1000 nM) were started 10 minutes prior to reducing the flow rate and were continued for the rest of the experiment.

Fig. 1

EFFECT OF RANOLAZINE ON THE VALUES OF LEFT VENTRICULAR MUSCLE pH BEFORE, DURING AND AFTER LOW FLOW GLOBAL ISCHAEMIA IN ISOLATED PERFUSED RAT HEARTS.
n= 7-10/GROUP



* Significantly different from controls. ($p<0.05$ Unpaired Student's t-test)

Results: Left ventricular muscle pH was decreased by 5 minutes after the start of low flow and returned to normal with restoration of flow. The pH of the ventricular muscle was on average lowest in the controls. The pH for the treated animals did not go as low as the controls. There was no difference in effect between the 1 nM and 10 nM concentrations and the 100 nM concentration had no apparent effect. Under the conditions of the assay, 1000 nM ranolazine prevented the ventricular pH from decreasing to the extent shown by the control samples. Use of a comparator compound such as a calcium channel blocker or opioid would strengthen the evidence that the effect is unique to ranolazine.

AT5697 In vitro effects of ranolazine (RS-43285-193) on K+ and lactate release during 15 minutes of 96% low flow global ischemia of rat hearts. April 1991.

Ranolazine was administered at concentrations of $1x10^{-6}$ and $1x10^{-5}$M to isolated paced Langendorff perfused rat hearts subjected to low flow global ischemia to study the possible effect on K+ and lactate release, considered indicators of ischemia. A 5 minute stabilization period was followed by 15 minutes of low flow. Coronary perfusate was collected just before start of low flow and at 3 minute periods throughout the low flow.

Results: Lactate efflux was decreased with the addition of ranolazine. K+ efflux was increased early on and decreased later. When the data was averaged into cumulative values, the ranolazine seemed to decrease K+ efflux.

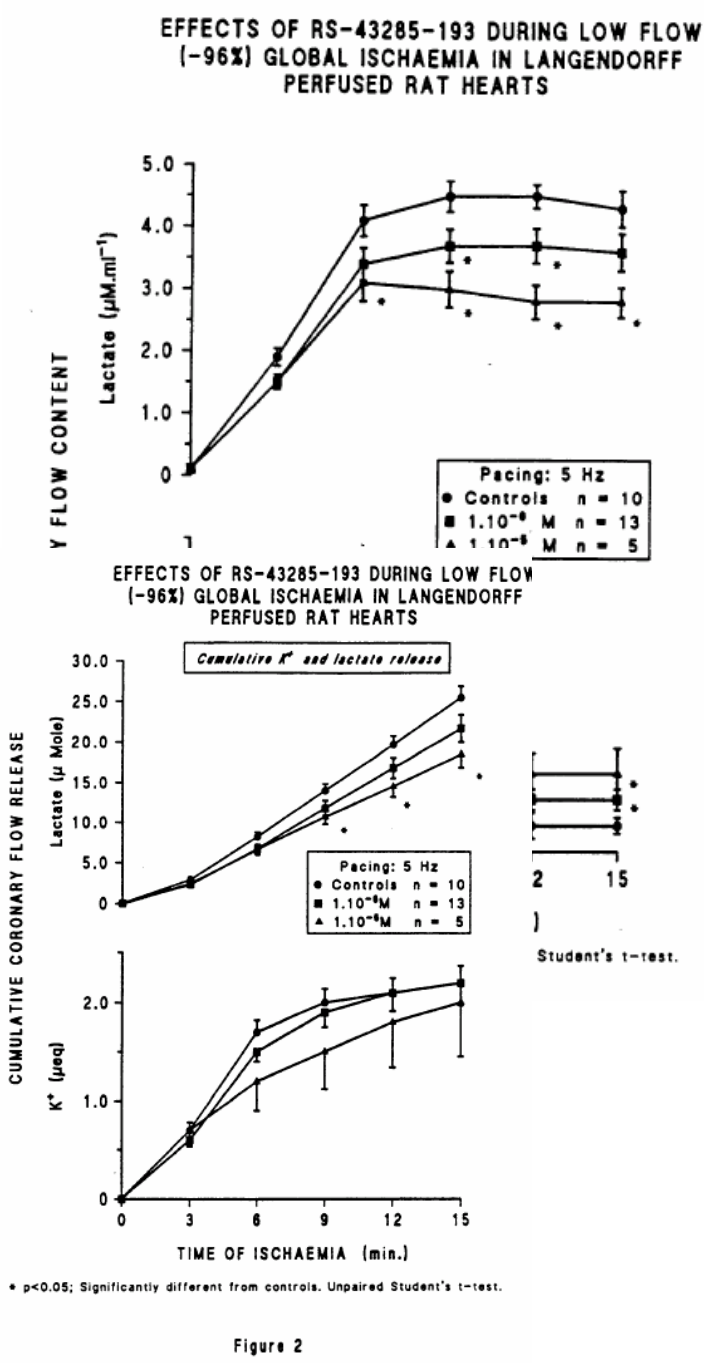


Figure 2

Under the conditions of the assay, the concentrations of ranolazine tested decreased lactate release. The addition of positive controls is desirable as is the addition of comparator compounds.

AT7009 Effects of ranolazine on high energy phosphate content and mitochondrial Complex I activity after global ischemia in isolated rat hearts. June 1995

Ranolazine was tested at 3µM and 10 µM in Langendorff-perfused rat hearts subjected to 20 or 30 minutes of global ischemia and 5 minutes of reperfusion. At the end of each experiment the hearts were freeze-clamped between pre-cooled aluminum plates and assayed for ATP and creatinine phosphate concentrations. In a separate series of perfusions the hearts were used to make mitochondria as described in another report.

Results: A table of results was presented pertaining to mitochondria. It is incompletely explained and subject to misinterpretation.The "effects of ranolazine on functional parameters and O2 consumption in normoxic working hearts" shows no difference between treated and control hearts. No positive controls were included. The graph of ATP content shows minimal differences between the doses at either time point. The graph of CP content showed a dose response at 30 minutes of ischemia. Use of standard deviations instead of standard error of the mean would increase confidence in the results.

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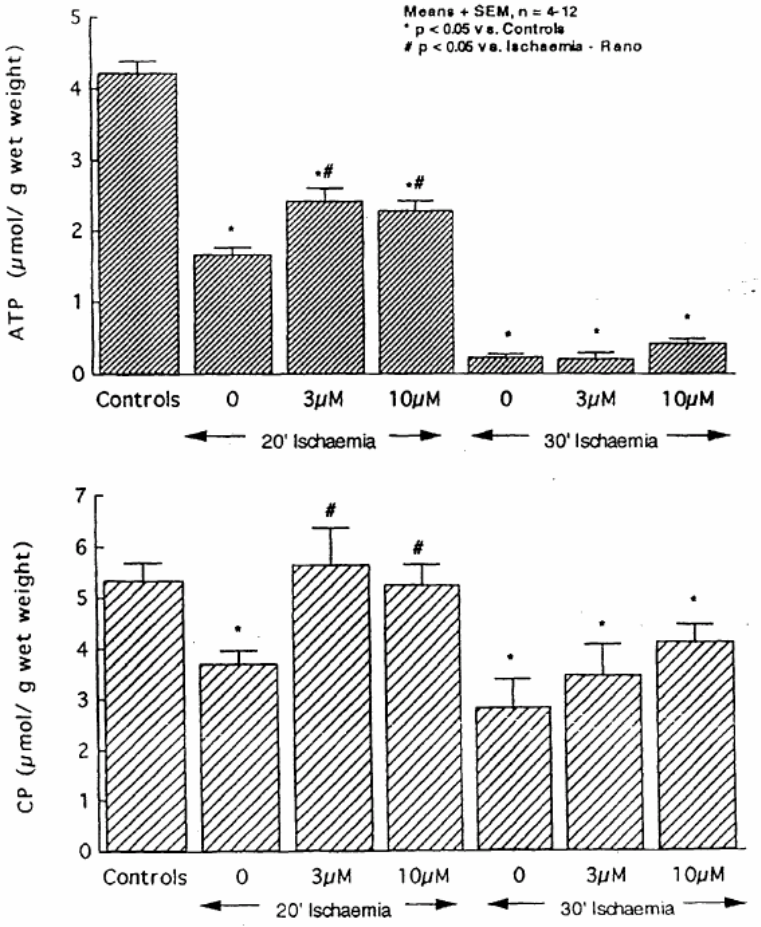
Figure 3 Effects of Ranolazine on the loss of high energy phosphates following global ischaemia in Langendorff-perfused rat hearts



AT6734 Cardioprotective effects of ranolazine (RS-43285) in the rabbit isolated perfused heart. June 1994

Hearts from male NZW rabbits were perfused via a Langendorff preparation at a constant flow. After equilibration, hearts were treated with either 10 or 20 µM ranolazine or vehicle for 10 minutes before exposure to a 30 minute period of global ischemia and 60 minutes of reperfusion. A normoxic control group was included. Aliquots of coronary effluent were collected from treated hearts at baseline, 15, 30, 45 and 60 minutes of reperfusion and analyzed for creatine kinase concentrations and K+ efflux. At the end of the experimental period hearts were analyzed for myocardial calcium and tissue ATP content.

Results: The presentation of results indicates that during the reperfusion, ranolazine-treated hearts 1)showed a dose-related decrease in EDP that progressed over time 2)showed a dose-related increase in LVD(developed)P vs control animals.

Creatine kinase increased in all groups, but to a lesser extent in the ranolazine animals. K+ increased very slightly in the control animals and stayed apparently constant in the treated animals. Tissue calcium increased over baseline in all groups, but to a lesser extent in the treated groups. Tissue ATP reached slightly greater (but statistically significant) levels compared to vehicle samples.

TEM was used to examine specimens from each group. Control hearts showed blurring of the myofibrillar z-bands, disrupted cristae, electron dense bodies that the sponsor said were suggestive of irreversible damage. Lanthanum chloride, used as an indicator of blood vessel integrity was scarce on the luminal surface of the vessels. The ranolazine-treated samples were reported to show few pathological changes. We are not told how samples were taken to ensure that they were representative.

The sponsor states that the mechanism by which ranolazine caused the reported changes is not known, but postulates that the results are consistent with what might be expected if the drug modulated anaerobic glycolysis.

Under the conditions of the assay, adding ranolazine to the isolated perfused hearts of healthy rabbits mitigated the effects of the low flow conditions.

AT7002 Protective effects of ranolazine (RS-43285) in isolated ginea[sic] pig hearts and their association with increases in active pyruvate dehydrogenase. Started January 1992- ended Jan. 1995

Perfused isolated hearts from female Duncan-Hartley guinea pigs were paced at 25% above the spontaneous rate (321±6 beats per minute) and electrical impulses delivered to the ventricles by implanted electrodes. A 30 minute equilibration period was followed by a 20 minute pre-treatment period ±ranolazine. The hearts were then perfused for another 30 minutes under low flow(0.7 ml/min) ischemic conditions ± ranolazine. Time-matched non-ischemic controls were used for comparison. Frozen hearts (not specified if they were from the first part of the study) were assayed for active, non-phosphorylated, pyruvate dehydrogenase (PDHa) and total PDH activity. Glycogen content and short-chain acylCoAs were also measured.

Results: PDHa was decreased compared to baseline both ± 10 mcM ranolazine but was

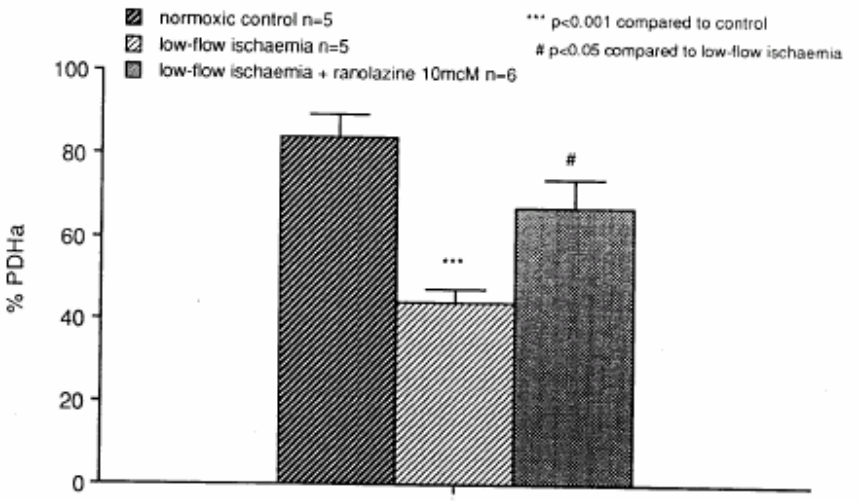
Table 1
Effects of ranolazine on tissue contents of short chain CoA esters and CoASH in low-flow ischaemic guinea pig hearts

Condition	Malonyl CoA	Acetyl CoA	Succinyl CoA	Glutathione CoA	CoASH	Acetyl CoA/ CoASH
Normoxic control	0.26 ± 0.03	1.35 ± 0.36	4.72 ± 0.52	1.69 ± 0.15	5.75 ± 0.50	0.27 ± 0.05
LFI control	0.47 ± 0.01	4.10 ± 0.09	2.49 ± 0.12	1.50 ± 0.17	5.04 ± 0.29	0.97 ± 0.04
LFI + 1 μM ranolazine	0.52 ± 0.03	4.05 ± 0.29	2.63 ± 0.38	1.35 ± 0.05	5.63 ± 0.54	0.86 ± 0.03
LFI + 10 μM ranolazine	0.41 ± 0.04	4.76 ± 0.62	3.18 ± 0.43	2.55 ± 0.42	5.73 ± 0.38	0.98 ± 0.09

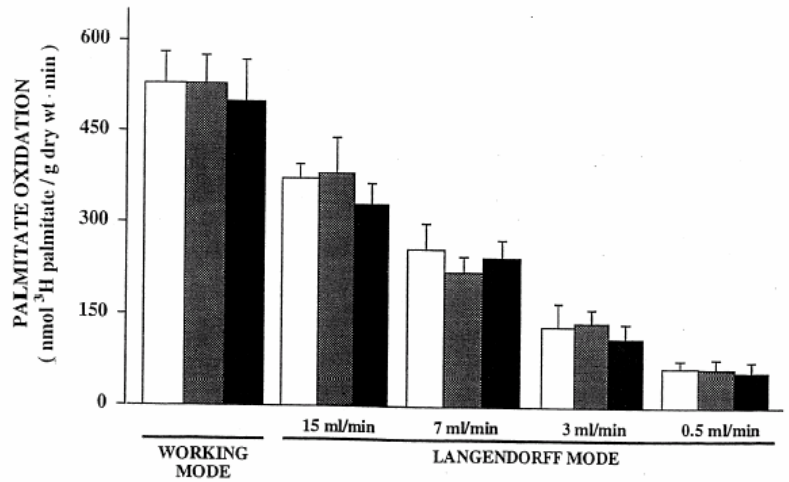
Values are nmol/g wet weight (or a ratio) and are given as mean ± s.e.m. for 5 (control, LFI control, LFI + 1 μM ranolazine) or 6 (LFI + 10 μM ranolazine) hearts. See text for statistical analyses.

decreased less in the presence of this one concentration of ranolazine. Lactate release did not differ between the controls and 1 μM ranolazine but was somewhat decreased with the 10 μM ranolazine. Lactate dehydrogenase (mIU.min.gm dry weight) did not differ between the two reported concentrations of ranolazine but both were lower values than the controls. CPK levels were highest for the untreated controls, followed by the 10 μM ranolazine and the lowest values shown by the 1 μM ranolazine samples. There does not appear to be any effect on short chain acyl CoAs.

Figure 1
The effect of 10 mcM ranolazine on tissue levels of activated pyruvate dehydrogenase (PDHa) after 30 mins low-flow ischaemia



Summary: The concentrations tested were not specified in the methods. Only 2 concentrations were presented, and in one assay critical to supporting the hypothesis only 1 concentration was presented (shown alongside). This study is not as convincing as it might be if more concentrations had been tested.

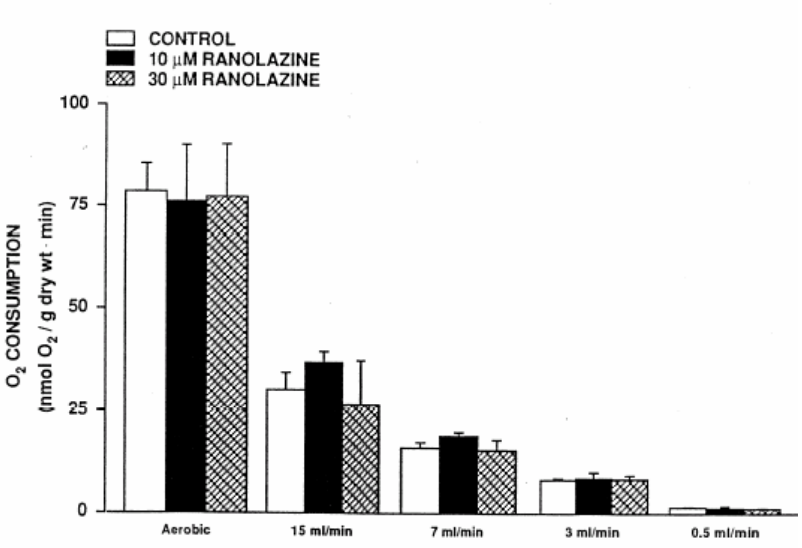


AT7004 Effects of ranolazine on substrate oxidation in isolated rat hearts. April 1993- January 1995.

Isolated rat hearts were perfused with Krebs-Henseleit buffer +3% albumin under conditions of normoxia, and on reperfusion after 30 minutes of no-flow ischemia and under conditions designed to give either low or high Ca (1.25 mM or 2.5mM),low or high FA (0.4 or 1.2 mM palmitate) ±

insulin. Hearts were either paced at 280 bpm or unpaced.

Glycolysis and glucose oxidation were measured by perfusing hearts with [5-^{3}H/U-^{14}C]glucose. Fatty acid oxidation was measured in separate experiments using either [1-^{14}C]palmitate or [9,10-^{3}H]palmitate. For low flow ischemia studies ranolazine was added to the perfusate at 1,10 or 30 μM. Steady state rates of glycolysis were determined by measuring tritiated water production as released at the enolase step after its separation from radiolabelled glucose.



Results: Palmitate oxidation was decreased in all groups over time. There was no apparent effect on palmitate oxidation with ranolazine treatment. There was no apparent difference between control and treated in terms of oxygen consumption.

The rate of glucose oxidation with 10 μM ranolazine was increased under most conditions. There was an apparent dose response in increased glucose oxidation with increasing concentration of ranolazine except under the lowest flow condition.

Isolated rat hearts, under some of the conditions studied, when treated with 1, 10 and 30 μM ranolazine showed a tendency to a dose-related increase in glucose oxidation. Glycolysis and palmitate oxidation were either unaffected or inconsistently affected.

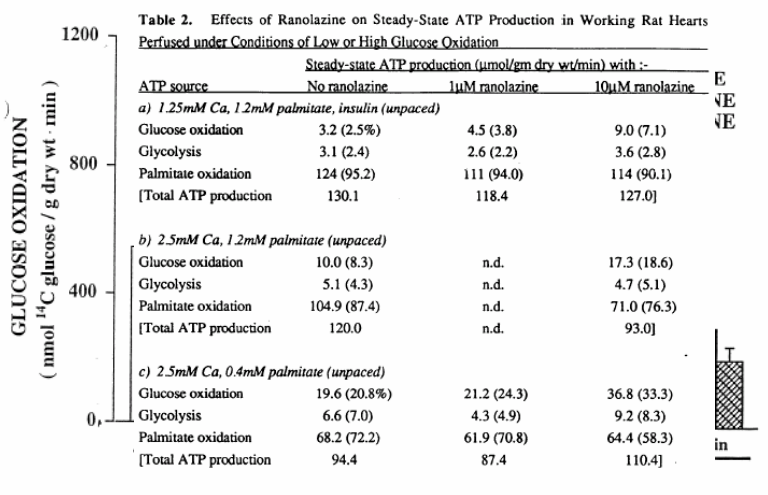


Table 2. Effects of Ranolazine on Steady-State ATP Production in Working Rat Hearts Perfused under Conditions of Low or High Glucose Oxidation

ATP source	Steady-state ATP production (μmol/gm dry wt/min) with :- No ranolazine	1μM ranolazine	10μM ranolazine
a) 1.25mM Ca, 1.2mM palmitate, insulin (unpaced)			
Glucose oxidation	3.2 (2.5%)	4.5 (3.8)	9.0 (7.1)
Glycolysis	3.1 (2.4)	2.6 (2.2)	3.6 (2.8)
Palmitate oxidation	124 (95.2)	111 (94.0)	114 (90.1)
[Total ATP production	130.1	118.4	127.0]
b) 2.5mM Ca, 1.2mM palmitate (unpaced)			
Glucose oxidation	10.0 (8.3)	n.d.	17.3 (18.6)
Glycolysis	5.1 (4.3)	n.d.	4.7 (5.1)
Palmitate oxidation	104.9 (87.4)	n.d.	71.0 (76.3)
[Total ATP production	120.0	n.d.	93.0]
c) 2.5mM Ca, 0.4mM palmitate (unpaced)			
Glucose oxidation	19.6 (20.8%)	21.2 (24.3)	36.8 (33.3)
Glycolysis	6.6 (7.0)	4.3 (4.9)	9.2 (8.3)
Palmitate oxidation	68.2 (72.2)	61.9 (70.8)	64.4 (58.3)
[Total ATP production	94.4	87.4	110.4]
d) 2.5mM Ca, 0.4mM palmitate, paced (280bpm)			
Glucose oxidation	51.2 (44.0%)	57.3 (44.2)	100.0 (64.8)
Glycolysis	10.3 (8.8)	10.9 (8.4)	11.7 (7.6)
Palmitate oxidation	54.9 (47.2)	61.3 (47.3)	42.7 (27.7)
[Total ATP production	116.4	129.5	154.4]

Data are calculated from the steady-state rates given in Table 1, from where n values and indication of error values can be obtained; n.d., not determined. Values in parentheses are calculated percentages from the μmol/gdw data shown; it should be noted that this only uses the measured parameters and does not include oxidation of endogenous substrates (for instance endogenous fat oxidation) [31], so that the total ATP production values given are therefore only derived from the exogenous sources measured.

In the tabular comparison of the high vs low calcium and palmitate, ±insulin and ± pacing, all of the many possible permutations of conditions were not examined. Also, discernible patterns of dose-response are not always apparent in the numbers. Another concentration of ranolazine would have been useful in this respect. There is no apparent effect on glycolysis or palmitate oxidation. There does appear to be an increase in glucose oxidation at the highest concentration of ranolazine. Table 4 shows the effects of 1 concentration of ranolazine added at reperfusion to hearts previously subjected to an ischemic period.

Table 4. Effects of ranolazine (10μM), added at reperfusion to hearts previously subjected to a 30min period of ischemia, on steady-state rates of glucose oxidation, glycolysis, and palmitate oxidation.

	Pre-ischemic	hearts (n) reperfused with :-	
Parameter	Control	No ranolazine	10μM ranolazine
glucose oxidation	337±36	377±66(8)	801±123(10)*@
glycolysis	3110±190	3570±440(8)	4440±380(10)@
palmitate oxidation	722±64	968±279(7)	566±108(8)

Hearts were perfused and rates determined as described in the legends to Figures 5 and 6. Rates shown are derived from the data shown in Figure 6 which has been averaged the last 30min period. *Indicates a significant effect of ranolazine compared to the appropriate "No ranolazine" value (unpaired t-test) and @indicates a significant difference from the appropriate pre-ischemic control value (paired t-test); the summed value for this latter value (i.e. all hearts) is shown in each instance.

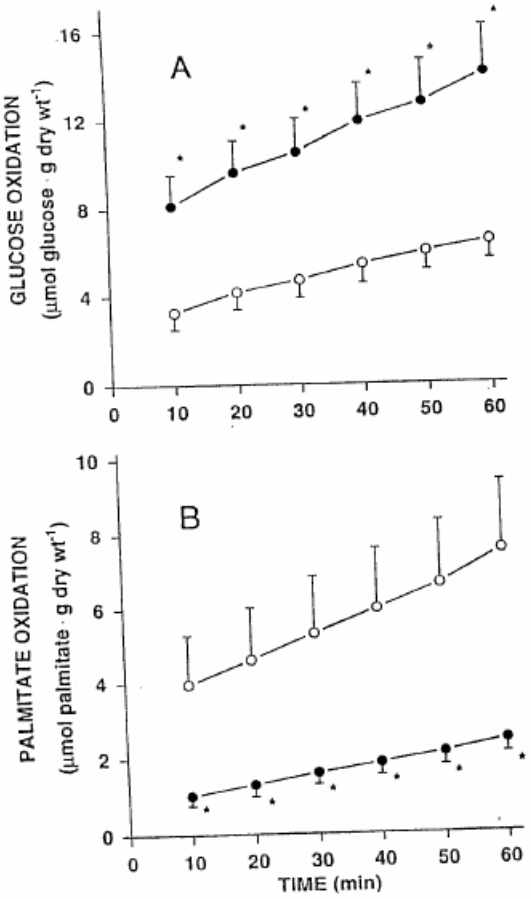
Under the conditions of the study, ranolazine produced an increase in glucose oxidation.

Effects on glycolysis and palmitate oxidation were inconsistent. The sponsor notes on p.283-284 of vol. 6 that calcium channel antagonists protect the heart by decreasing workload and metabolic energy demand.

AT7005 Effects of ranolazine on energy utilisation in skeletal muscle. Sept. 1994-February 1995

The "epitrochlearis" muscle was isolated from rats and superfused with oxygenated Krebs buffer containing 3% albumin + 0.4 mM palmitate, 5.5 mM glucose, 0.5 mM lactate + a "physiologic" amino acid mixture and either [5-^{3}H/U-^{14}C]glucose to measure glucose oxidation and glycolysis or [9,10-^{3}H]palmitate and [U-^{14}C]lactate to measure palmitate and lactate oxidation ±10µM ranolazine for up to 1 hour following a 5-10 minute pre-equilibration period. Contralateral muscles from the same animal were used.

Oxygen consumption was monitored under several flow conditions. Samples were analyzed for distribution of acetyl CoA carboxylase isoforms. Short chain CoA esters were extracted and analyzed by HPLC.



Results: 10µM ranolazine-treated muscle preps showed somewhat greater rates of glucose oxidation and glycolysis than the untreated preps. Lactate oxidation was unchanged and palmitate oxidation decreased (closed circles in the graph are + ranolazine). There was no significant difference between control and treated regarding tissue contents of CoA, acetyl CoA, succinyl CoA and malonyl CoA.

Under the conditions of the study, the one concentration of ranolazine tested on isolated rat skeletal muscle decreased palmitate oxidation and increased glucose oxidation. Oxygen consumption was slightly (NS) decreased relative to the controls. A dose-response effect would strengthen the study as would the use of comparator compounds.

CL7069 Ranolazine effects on pyruvate dehydrogenase activity in perfused normoxic rat hearts: evidence for enzyme activation by an indirect mechanism. Jan 1994- February 1995 Reported June 1995

Hearts were collected from male Wistar rats and perfused with Krebs-bicarbonate buffer. Hearts were perfused with Krebs-bicarbonate buffer and one of the following concentrations of palmitate (0,0.4, 0.8 or 1.2 mM). In some cases the palmitate was replaced with either octanoate or acetate (±albumin). Hearts were pre-perfused for 10 minutes without palmitate, octanoate or acetate then for 30 minutes with palmitate (0, 0.4, 0.8 or 1.2 mM) ±20 μM ranolazine and/or 1mM dichloroacetate (DCA, a pharmacological analogue of pyruvate).In some cases the palmitate was replaced with either octanoate or acetate ±albumin. Cardiac function and heart rate were continuously monitored by an isometric tension device. At the end of the perfusion period the hearts were freeze clamped and stored in liquid nitrogen until analysis.

Cardiac mitochondria were prepared from freshly euthanized rats. Mitochondria were incubated in buffer with respiratory substrates (10mM 2-oxoglutarate, 0.5mM malate), ± ranolazine, for 4 minutes. Uncoupled mitochondria were not given the respiratory substrates; 1μM FCCP, Mg.ATP and oligomycin were added instead.
Initial pyruvate dehydrogenase activity (PDHa) was assayed spectrophotometrically from disrupted mitochondrial pellets. Total PDH was assayed after conversion of all the enzyme into the active form using pig heart phosphate phosphatase. PDHa kinase was assayed either by incorporation of ^{32}P from [γ-^{32}P]-ATP into purified bovine or human PDH. Glutamate dehydrogenase was assayed as an alternative mitochondrial marker and PDHa expressed over this activity. PDHa kinase was assayed either by following incorporation of 32P from [γ-32P]ATP into purified bovine or human PDH or by following rate of inactivation of pig heart PDH. Unspecified powdered tissue was analyzed for CoA esters and free CoA.

Results:
Ranolazine inconsistently affected PDHa content in the presence of varying concentrations of palmitate. Ranolazine + DCA (a known PDHa stimulator) produced results different from DCA or ranolazine alone.

PDHa/GDH content of hearts perfused with :-

[palmitate]	No additions	20μM ranolazine	1mM DCA	ranolazine + DCA
0mM	0.21 ± 0.03	0.19 ± 0.03	0.42 ± 0.03*	0.52 ± 0.02*
0.4mM	0.08 ± 0.01	0.18 ± 0.02*	0.25 ± 0.02*	N.D.
0.8mM	0.09 ± 0.03	0.18 ± 0.02*	0.32 ± 0.02*	0.44 ± 0.01*†
1.2mM	0.08 ± 0.03	0.08 ± 0.02	0.13 ± 0.02	0.33 ± 0.03*†

Hearts were perfused (plus 3% albumin) for 30min under the conditions shown (see Methods for full details). PDHa is expressed as a ratio of the glutamate dehydrogenase (GDH) activity; this was not altered by any of the conditions tested and was in the range 6-10units/g wet weight. Similar results were obtained if PDHa was expressed over total PDH activity (not shown); this was also not altered by any of the conditions tested and was in the range 3-6units/g wet weight. The average PDH(total)/GDH ratio was 0.62 ± 0.05 from all hearts. N.D., not determined. Results are means ± s.e.m. for at least 4 hearts. *Significantly different from no addition; †significantly different from ranolazine or DCA alone.

Table 3. Effects of ranolazine and other agents on the steady-state PDHa content of isolated rat heart mitochondria

Other conditions	steady-state PDHa/GDH content of mitochondria incubated with :- no ranolazine	100μM ranolazine
Control, coupled	0.03 ± 0.01	0.03 ± 0.01
1mM DCA	0.15 ± 0.03*	0.16 ± 0.04*
0.2mM pyruvate	0.18 ± 0.04*	0.17 ± 0.03*
50nM free Ca^{2+}	0.16 ± 0.02*	0.18 ± 0.02*
50nM Ca^{2+} + 1mM DCA	0.30 ± 0.03*†	0.31 ± 0.04*†
10μM palmitoyl carnitine	0.01 ± 0.003	0.01 ± 0.003
palmitoyl carnitine + 1mM DCA	0.11 ± 0.02*†	0.12 ± 0.03*†
10mM L-carnitine	0.20 ± 0.04*	0.21 ± 0.05*
100μM DCA	0.08 ± 0.01*	0.08 ± 0.01*
1mM L-carnitine	0.07 ± 0.01*	0.07 ± 0.01*
100μM DCA + 1mM carnitine	0.14 ± 0.01*†	0.14 ± 0.02*†
Control, uncoupled	0.15 ± 0.04	0.15 ± 0.03
1mM DCA	0.28 ± 0.04*	0.30 ± 0.04*

See Methods for full details of incubations which were for 4min, but essentially similar results were obtained after 8min; in incubations up to 30min ranolazine did not give values any different from controls. GDH activity was not altered by any conditions tested and averaged 153 ± 12 mU/mg protein. Total PDH activity (not shown) was not always measured but also did not change and averaged 109 ± 9 mU/mg protein. Results are means ± s.e.m. of values obtained from at least 3 different preparations. *Significantly different from appropriate control; †significantly different from either agent alone (i.e. effect of DCA). Ranolazine had no significant effects at this concentration or at any other over the range 0.0001-1mM; in contrast, DCA, pyruvate, carnitine and Ca^{2+} showed concentration-dependent effects (not shown).

Table 2. Effects of pyruvate, DCA and ranolazine on PDHa kinase activity.

	Kinase activity (% control) in the presence of :-	
Conditions	No ADP	0.5mM ADP
Control	(100)	94
1mM ranolazine	103	99
5mM DCA	71	42
20mM DCA	56	35
4mM pyruvate	90	35

Activity was measured by following the incorporation of ^{32}P from [γ-^{32}P]-ATP into PDH (see Methods); essentially similar results were obtained when activity was measured by following changes in amounts of PDHa (see Methods). A typical experiment is shown; this was repeated on 3 separate occasions. Ranolazine had no effects over the range 0.001-1mM. The enhancement of the effects of DCA and pyruvate by ADP is consistent with the results of Pratt and Roche (1979).

Lack of effect of ranolazine on PDH or its interconverting enzymes

To bring about increases in PDHa within the tissue, either PDH phosphate phosphatase must be activated and/or PDHa kinase must be inhibited. We examined the effects of ranolazine on these two enzymes using purified preparations (see Methods), and in comparison to effects of known regulators (see Introduction). For completeness, we also studied the catalytic activity of PDH. In all these experiments no evidence for any direct effect of ranolazine on the PDH

system was found. A typical example is shown in Table 2 for PDHa kinase. Here it is seen (see also legend) that the inhibitory effects of pyruvate and DCA (and ADP) on this enzyme are all readily evident, whereas ranolazine, up to 1mM, had no effects at all. In the case of the phosphatase, the activatory effects of Mg^{2+}, Ca^{2+} and spermine (see Introduction) were all readily evident but again no effects of ranolazine (0.001 - 1mM) were observed (not shown). There were also no effects of ranolazine (up to 1mM) on the catalytic activity of PDH itself; this was the case in terms of the enzyme's Vmax or Km for any of its substrates (pyruvate, CoA or NAD^+), and for the Ki of its end-product inhibitors NADH and acetyl CoA (not shown).

There remained the possibility that some other mitochondrial co-factor was required for ranolazine to have an effect on an enzyme of the PDH system, and therefore an extensive series of experiments was conducted with isolated rat heart mitochondria which contain all of these enzymes. However, the results in Table 3 (see also legend) show that ranolazine had no effect on the steady-state PDHa content of rat heart mitochondria incubated under a variety of conditions. In contrast, known regulators of the kinase (DCA, pyruvate) and phosphatase (Ca^{2+}) again had the expected effects (Table 3) (see McCormack *et al.*, 1982). Ranolazine also had no effect in the presence of these other effectors, even though additive effects of e.g. DCA and Ca^{2+} (as an effector of the kinase plus an effector of the phosphatase) were readily evident. This suggests that ranolazine does not affect either PDH kinase or phosphatase directly to bring about its effects on PDHa. However, PDHa can be altered indirectly by events leading to changes in the matrix content of effectors of these enzymes. This is shown in Table 3 in the case of carnitine which has no direct effects on the enzymes, but causes a reduction in the matrix acetyl CoA/CoA ratio (Lysiak *et al.*, 1988) and in this way leads to kinase inhibition.

The sponsor postulates that while ranolazine has no direct effect on PDH kinase or phosphatase, the drug may cause a decrease in mitochondrial acetyl CoA content which will lead to a decrease in kinase activity. A decrease in Acetyl CoA will also decrease end-product inhibition of the catalytic activity of PDH.

CVT303.021-N: Attempt at optimization of the energy balance of the cardiac myocyte by the synergistic action of a dietary fatty acid and a cytoprotective pharmaceutical agent.1998

This report is marked as a "training thesis" and is not completely translated from the original French. Cultured neonatal rat cardiomyocytes were used to assay $^{14}CO_2$ production from 3 substrates: palmitate, glucose and octanoate. Cellular contraction was also monitored and quantitated. The method used contains various original components such as an electronic collimator and original software for processing the signal to establish contraction rhythm. This equipment was used to evaluate the contraction rhythm before the study to select cultures with a spontaneous rhythm of 90-150 contractions per minute. The set-up was also used to evaluate the influence of treatments on the cellular rhythms. Unfortunately, no form of validation or standardization data was presented for this apparatus.

Results: Addition of either trimetazidine or ranolazine decreased the contraction rate of the myocardial cells. (p.26/318). Both drugs seemed to decrease $^{14}CO_2$ production from palmitate to the same extent. Neither seemed to have a significant effect when the substrate was octanoate. There were slight increases in CO2 production compared to "temoin" from both drugs, a greater increase from ranolazine, when the substrate was glucose. These differences are marked as statistically significant. Under the conditions of the study, the addition of ranolazine to the cultures decreased CO2 production when compared to the control cultures with a substrate of palmitate, increased CO2 production when the substrate was glucose and was no different than control when the substrate was octanoate.

AT5450 The binding of [^{3}H]-RS-43285-193 to rat cardiac mitochondria. February 1987-August 1989.

Hearts were collected from rats and mitochondria prepared. The mitochondria were incubated with tritiated ranolazine. In competitive binding experiments compounds were present over the concentration range of 10^{-4} – 10^{-10}M. Incubations were carried out at 25ºC for 45 minutes. The inhibition of specific binding of [^{3}H]-RS-43285-193 was determined in the presence of 10µMRS-87505 or 10 µMRS-88216. These compounds were undefined.

Results: The sponsor reported that radiolabelled Ranolazine associated to rat mitochondria with a $t_{1/2}$ for association of 2.5 minutes. Binding reached equilibrium within 10 minutes and was stable for another 40 minutes. The addition of unlabelled RS-43285 caused a rapid dissociation

of the labelled material. The addition of KCN decreased the binding/association of ranolazine with the mitochondria to background levels. The process was inhibited by KCN (suggesting energy dependence).

Under the conditions of the study, [^{3}H]-RS-43285-193 rapidly associated and dissociated from isolated rat cardiac mitochondria. The binding was non-saturable, low affinity and unlikely to be a classical receptor. The isomers were reported to be equipotent at binding in this system, indicating a lack of stereospecificity.

AT6052 Studies of ^{3}H-ranolazine (RS-43285-193) uptake by isolated rat hearts during normoxic perfusion according to Langendorff. October 1990-December 1990.

Tritiated ranolazine at concentrations from 0.814 x 10^{-9}M (18.7μCi.l^{-1}) to 16.34 x 10^{-9}M (374 μCi.l^{-1})was infused into isolated rat hearts perfused under normoxic conditions according to the methods of Langedorff. The 25 minute perfusion followed a 5 minute stabilization period. After the perfusion, mitochondria were isolated and the radioactivity measured.

Results: The amount of radioactivity administered vs the amount of radioactivity found in the mitochondria and/or in the whole heart was linear. The sponsor proposed that this indicated a lack of mitochondrial accumulation. The conclusion is thus that there is a linear relationship between amount of radioactivity perfused through the hearts and that recovered from the tissue.

CVT303.007-N Effect of ranolazine on fatty acid β oxidation in rat heart mitochondria, part I. Sept 1996- March 1997.

Rat heart mitochondria were prepared from male Sprague-Dawley rats. The fatty acid oxidation of [1-^{14}C]palmitoylcarnitine ± ranolazine was assayed by liquid scintillation counting. Oxygen uptake by the rat heart mitochondria ± inhibitor ± palmitoyl carnitine or acyl CoA was measured by an oxygen electrode.
Ranolazine was added to the incubation mixtures as an ethanolic solution. The sponsor states that the amount of ethanol used did not affect the rate of fatty acid oxidation but does not present data to support this.
The effect of ranolazine on fatty acid oxidation was studied with purified enzymes of β-oxidation. Ten β-oxidation enzymes catalyzing 4 types of reactions were assayed ± 30 or 100 μM ranolazine.
Results: It was unknown how rapidly ranolazine was taken into mitochondria so in a preliminary experiment, rates of β-oxidation were determined after preincubating mitochondria with ranolazine for different periods of time. Inhibition was optimal without first incubating the mitochondria with the drug. The β-oxidation capacity of the mitochondria decreased significantly during the preincubation time, therefore, mitochondria were not preincubated with ranolazine in subsequent experiments.

Table 3
Effect of Ranolazine on the Rates of Myocardial Respiration Supported by Palmitoyl-L-carnitine, Palmitoyl-CoA, Linoleoyl-CoA, or Pyruvate

Substrate	Ranolazine (μM)	Respiration Rate (nmol O/min/mg)	Inhibition (%)
Palmitoylcarnitine	0	196 ± 4.9 (3)	0
	30	173 (1)	12
	100	132 ± 1.4 (3)	33
Palmitoylcarnitine	0	191 ± 1.2 (3)	0
	30	168 ± 1.1 (4)	12
	100	134 ± 4.2 (3)	30
Palmitoyl-CoA	0	96 ± 1.1 (3)	0
	30	66 ± 0.5 (3)	31
	100	47 ± 0.7 (4)	52
Linoleoyl-CoA	0	100 ± 1.1 (3)	0
	30	89 ± 1.2 (3)	12
	100	62 ± 1.2 (3)	38
Pyruvate	0	193 ± 1.2 (3)	0
	30	189 ± 2.2 (3)	2
	100	184 ± 2.4 (3)	5

The data is presented as "Inhibition of myocardial β-oxidation by ranolazine as a function of the preincubation time." Mean without SD was presented.

Ranolazine was tested in a concentration range from 1-100 μM. The acid soluble products (nmol/min/mg protein) decreased from the control value of 1.99± 0.04 to 1.43±0.05 at 100μM ranolazine (28% inhibition) . A repeat experiment produced a control value of 2.74±0.06 and the high dose drug concentration gave a value of 1.64±0.05 (40% inhibition).

Two reported concentrations of ranolazine caused some inhibition of respiration with different substrates.

Ranolazine had essentially no effect on the mitochondrial enzymes of β-oxidation. There was a slight effect on long-chain enoyl-CoA hydratase and an even smaller effect on enoyl-CoA hydratase (crotonase). The sponsor stated that inhibition of crotonase was likely to be the cause of impaired fatty acid oxidation in the presence of ranolazine and therefore was further investigated. It was their interpretation of further studies that the inhibition was non-competitive with a Ki of 180 μM.

Effect of Ranolazine on the Activities of Mitochondrial Enzymes of the β-Oxidation Spiral

Enzyme	Ranolazine (μM)	Activity (Units/mg)	Inhibition (%)
Short-chain acyl-CoA dehydrogenase	0	0.79 ± 0.03 (6)	0
	30	0.78 ± 0.02 (6)	0
	100	0.77 ± 0.01 (7)	0
Medium-chain acyl-CoA dehydrogenase	0	2.2 ± 0.02 (6)	0
	30	2.2 ± 0.02 (7)	0
	100	2.2 ± 0.06 (6)	0
Long-chain acyl-CoA dehydrogenase	0	2.1 ± 0.01 (6)	0
	30	2.1 ± 0.03 (7)	0
	100	2.1 ± 0.04 (6)	0
Very long-chain acyl-CoA dehydrogenase	0	0.74 ± 0.01 (6)	0
	30	0.79 ± 0.02 (6)	0
	100	0.77 ± 0.03 (6)	0
Enoyl-CoA hydratase (crotonase)	0	2920 ± 70 (6)	0
	30	2550 ± 50 (6)	13
	100	2120 ± 60 (6)	27
Long-chain enoyl-CoA hydratase	0	10.9 ± 0.2 (4)[1]	0
	30	9.5 ± 0.3 (7)[1]	13
	100	6.9 ± 0.3 (5)[1]	37
3-Hydroxyacyl-CoA dehydrogenase	0	323 ± 4 (6)	0
	30	340 ± 5 (6)	0
	100	350 ± 6 (6)	0
Long-chain 3-hydroxyacyl-CoA dehydrogenase	0	12.2 ± 0.1 (7)[1]	0
	30	12.7 ± 0.4 (6)[1]	0
	100	13 ± 0.2 (6)[1]	0

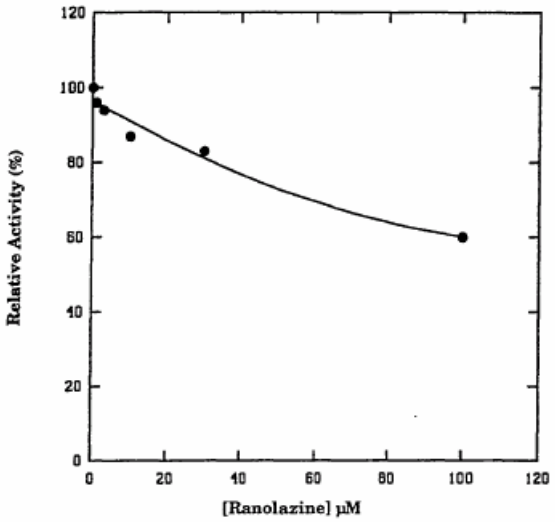


Fig. 1. Inhibition of β-oxidation by ranolazine in isolated rat heart mitochondria. [1-^{14}C]palmitoyl-L-carnitine served as the substrate.

CVT303.008-N Effect of ranolazine on fatty acid β-oxidation in rat heart mitochondria, part 2. September 1997-December 1997, Reported Jan.1998.

Mitochondria were isolated from male rat hearts and loaded with carnitine by incubation. The assay mixture contained buffer, rotenone, antimycin A and the carnitine-loaded mitochondria. In control experiments, mersalyl acid was added to inactivate the translocase. The uptake of carnitine was initiated by the addition of 0.5mM [^{3}H-methyl]-L-carnitine to the assay mixture. The reaction was stopped by acidification and centrifugation. Enoyl CoA hydratases were assayed for activity as was carnitine palmitoyltransferase (CPT) and carnitine:acylcarnitine translocase(CAT).

Results: There was no drug effect on CPTII. The Triton X-100 used in the assay inactivates CPT I so it was assumed that measured activity was for CPTII. There was a dose-related decrease in CAT activity.

Table 2
Effect of Ranolazine on the Activity of Carnitine: Acylcarnitine Translocase

Experiment	Ranolazine (μM)	Activity (dpm/10 min)	Inhibition (%)
I	0	1,460 ± 50 (5)	0
	30	1,140 ± 40 (5)	22
	100	920 ± 70 (5)	37
	300	430 ± 70 (5)	71
II	0	1,400 ± 50 (5)	0
	30	1,130 ± 20 (5)	19
	100	750 ± 50 (4)	46
	300	530 ± 70 (5)	62

There was a dose-related decrease in mitochondrial respiration when the substrates provided were either palmitoylcarnitine (15% and 35% less than 0 mM ranolazine) or octanoate (28% and 39% less than 0 mM ranolazine).

It was mentioned in the results that R- and S-ranolazine produced essentially identical results to the racemate regarding the inhibition of long chain enoyl CoA hydratase (Ki values of 0.195mM, 0.19 mM and 0.19mM respectively). Non-competitive inhibition was again reported.

CVT303.013-N Effect of ranolazine on fatty acid β-oxidation in rat heart mitochondria, Part 3. December 1998- December 1999.

Carnitine:acylcarnitine translocase was assayed by measuring uptake of radioactive L- carnitine in isolated rat liver mitochondria preloaded with L-carnitine. (R-) and (S-) ranolazine were both evaluated for effects on β-oxidation of palmitoyl-L-carnitine in coupled rat heart mitochondria. Results: Both enantiomers inhibited β-oxidation. At concentrations of 0.1mM and 0.3 mM, (R)-ranolazine was twice as effective as the (S-) isomer while at the lowest concentrations there was no apparent difference.

differences were detected. Inhibitions of 28% and 40% previously observed with 0.1 mM racemic ranolazine (1) are similar to the level of inhibition caused by the S-isomer.

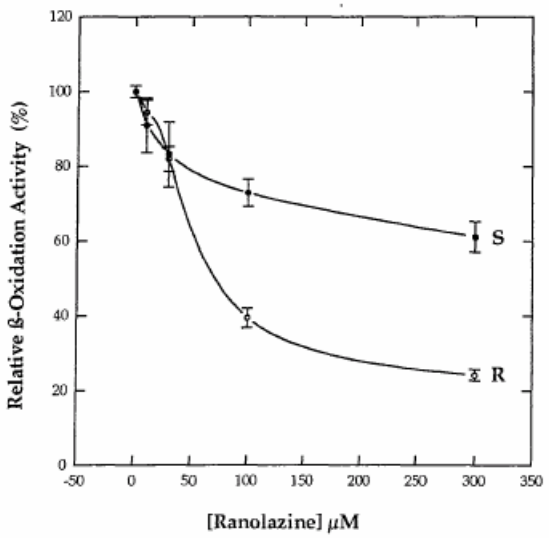


Fig. 1. Effects of (R)- and (S)-ranolazine on the ß-oxidation of[1-^{14}C]palmitoyl-L-carnitine in rat heart mitochondria. For further details see Attachment 1.

Neither enantiomer nor the racemic mixture showed any effect in this system on the β-oxidation of octanoate. Both isomers showed an inhibition of carnitine:acylcarnitine translocase

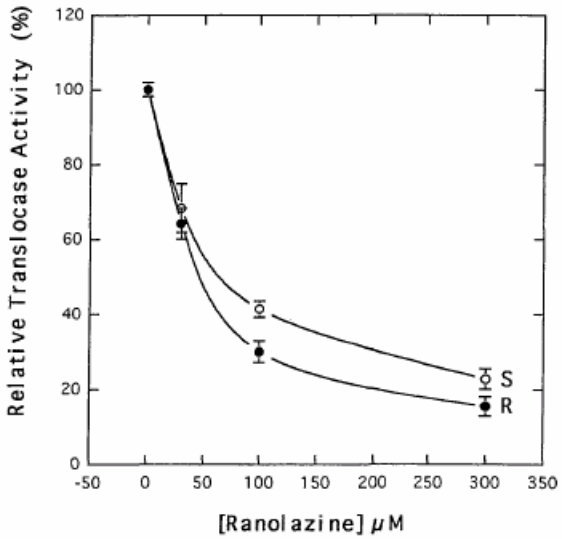


Fig. 2. Effects of (R)- and (S)-ranolazine on the activity of carnitine:acylcarnitine translocase in rat liver mitochondria. For detailed data see Attachment 2.

Effect of Ranolazine on Carnitine:Acylcarnitine Translocase of Rat Liver Mitochondria

Mersalyl (μM)	Ranolazine S-, or R-Isomer (μM)	Carnitine:Acylcarnitine Translocase Activity (dpm/5 min) orig. count	(count-backgrd[1)]	Rel.Act. (%)	Inhibition (%)
None	None	2100 ± 40 (5)	1170	100 ± 2	0
4	None	940 ± 45 (5)			
None	S- 30	1740 ± 140 (4)	800 §	68 ± 6	32
None	S- 100	1430 ± 46 (5)	490 §	42 ± 2 *	58
None	S- 300	1210 ± 57 (5)	270 §	23 ± 3 *	77
None	R- 30	1700 ± 86 (4)	750 §	64 ± 4	36
None	R- 100	1290 ± 60 (5)	350 §	30 ± 3 *	70
None	R- 300	1130 ± 55 (4)	190 §	16 ± 3 *	84

1 Counts corrected for non-specific uptake determined in the presence of the translocase inhibitor mersalyl.
§ Rate of translocation is different from the rate observed in the absence of mersalyl at $P < 0.001$.
* Inhibition of translocation by S-isomer is different from the inhibition by the R-isomer at $P < 0.01$

When comparing the two enantiomers of ranolazine, the (R) isomer is the more effective inhibitor of both ß-oxidation and translocase. However, the patterns of inhibition obtained with (S)-ranolazine for ß-oxidation and translocase differ significantly from each other, whereas the responses of ß-oxidation and translocase to the (R) isomer are very similar. This imperfect relationship between the inhibitions of ß-oxidation and translocase may be an artifact attributable to the translocase assay. Use of an assay method that yields initial velocities may result in a better correlation and improve the confidence about the cause-effect relationship between the ranolazine-induced inhibitions of translocase and ß-oxidation.

Effect of Ranolazine on the ß-Oxidation of [1-^{14}C]-Palmitoyl-L-carnitine by Rat Heart Mitochondria

Ranolazine Isomer	Concn. (μM)	Acid Soluble Products (nmol/min/mg)	Rel. Activity (%)	Inhibition (%)
R-isomer	None	1.78 ± 0.03 (13)	100.0 ± 1.6	0
	10	1.68 ± 0.06 (5) §	94.4 ± 3.3	5.6
	30	1.46 ± 0.06 (6) ‡	82.0 ± 3.3	18.0
	100	0.70 ± 0.05 (8) ‡	39.5 ± 2.6 *	60.5
	300	0.43 ± 0.03 (9) ‡	24.2 ± 1.5 *	75.8
S-isomer	None	1.78 ± 0.03 (13)	100.0 ± 1.6	0
	10	1.62 ± 0.13 (8) §	91.0 ± 7.3	9.0
	30	1.48 ± 0.16 (6) ‡	83.2 ± 8.7	16.8
	100	1.30 ± 0.06 (7) ‡	72.9 ± 3.6 *	27.1
	300	1.09 ± 0.07 (12) ‡	61.1 ± 4.1 *	38.9

* Inhibition of ß-oxidation by the R-isomer is different from the inhibition by the S-isomer at $P< 0.001$.

Rate of ß-oxidation is different from the rate observed in the absence of the inhibitor at $P< 0.005$ (§); at $p< 0.001$ (‡).

AT5184 Effects of ranolazine on mitochondrial function: Respiration, calcium uptake and release and carnitine palmitoyl transferase activity. April 1989 – March 1990.

Cardiac mitochondria were isolated from male Sprague-Dawley rats. Mitochondrial NADH-Cytochrome c reductase activity was determined by spectrophotometric methods. Mitochondrial OGDH (oxoglutarate dehydrogenase)activity was determined by fluorimetry. To determine the rate of efflux of calcium from the mitochondria, 200 nM free calcium was added to the mitochondrial mix until formation of NADPH reached equilibrium. Efflux of calcium was initiated by the addition of 10 nM NaCl and the decay in the absorbance signal (340 nM) followed for 5 minutes.

CPT1 activity was measured in mitochondria isolated from Sprague-Dawley rats by measuring the formation of palmitoyl [^{3}H]-carnitine from palmitoyl-CoA and L-[^{3}H]-carnitine.

Results:
In the textual summary of results, it was reported that tight coupling of the respiration and oxidative phosphorylation within the mitochondria were evidenced by the need to add 180mcM ADP to the malate/glutamate containing preps. The respiratory control ratio (state 3/state 4 respiration) was decreased in the presence of $1x10^{-3}$ and $1x10^{-4}$M ranolazine but not with $1x10^{-5}$ M ranolazine. Ranolazine inhibited oxidation of NADH with a lower affinity than that of rotenone (pki 4.24±0.09 vs pki 8.31±0.10 for rotenone). It was also stated that mitochondrial CPT1 was inhibited by ranolazine but only at high concentrations.
A table labelled "Effect of ranolazine on malate/glutamate stimulated respiration" is without legend, concentrations without units are given and as such the whole is uninterpretable. Table 2 lists 3 compounds and gives a pKi value, presumably for Cytochrome c reductase activity. All tables are without legends. We don't know how many concentrations of ranolazine were tested or what those concentrations were. There is a mention of diltiazem being used to inhibit the calcium dependent stimulation of NADH-OGDH as a comparator compound. Again, the number of concentrations tested and what those concentrations were could not be located in the report. The report does not provide enough material for the reviewer to come to an independent conclusion.

AT7037 Effects of ranolazine on the mitochondrial beta-oxidation pathway in vitro. January 1995- October 1995.

Mitochondria were prepared from rat heart and skeletal muscle. Radiolabelled palmitate was added and terminated at 0, 4 or 10 minutes for samples for the measurement of acyl carnitine intermediates. Beta-oxidation flux was determined in a separate experiment by measurement of the acid-soluble radioactivity generated from the labelled palmitate. Ranolazine was tested at 30μM and 100 μM.

Effects of ranolazine on flux through the beta-oxidation pathway of rat skeletal muscle mitochondria

Condition	Rate of fatty acid oxidation
H_2O control	30.7
Placebo control	30.6
30 μM ranolazine	18.5
100 μM ranolazine	16.5

Rates are given as nmol C_2 units/min/ml and have been calculated over the 12 min period of incubation (see Methods). Rates obtained were linear (see below). (Samples were collected at 0, 4, 8 and 12 min).

Results: It was reported that the isolated rat heart mitochondria had too high activities of both ATPase and acyl CoA hydrolase to "make the assay feasible." It was therefore decided to assess the actions of the drug using rat skeletal muscle mitochondria.
Ranolazine decreased the flux through the β-oxidation pathway. There was little difference in effect between the two concentrations.

Ranolazine had some effect on the C2 and C4 acyl carnitine intermediates after 4 minutes of incubation. A difference was also noted at 10 minutes. The sponsor's comment with the 10-minute table is noteworthy.

Table 3

Effects of ranolazine on acyl carnitine intermediates after 4 min incubation of rat skeletal muscle mitochondria

	Content of acyl Cn (nmol/ml) in samples treated with:-			
Acyl Cn	Placebo	Blank	30 µM ranolazine	100 µM ranolazine
C2	204.3	226.2	132.1	144.7
C4	1.7	1.9	1.2	0.9
C6	0.9	1.2	0.8	0.6
C8	0.3	0.4	0.5	0.5
C10	0.3	0.5	0.4	0.3
C12	0.6	0.7	0.7	0.4
C14	0.4	0.4	0.5	0.3
C16	5.9	8.0	9.0	8.0
C16:1	0.21	0.35	0.31	0.45
C16:OH	0.14	0.16	0.13	0.16

* These samples have been ignored for the analyses of the results. They are inconsistent with the results in Table 3 (as more C2 build-up is expected with time) and with the flux results (Table 1). The reason why these samples gave erroneous results was not found out.

Effects of ranolazine on acyl carnitine intermediates after 10 min incubation of rat skeletal muscle mitochondria

	Content of acyl Cn (nmol/ml) in samples treated with:-			
Acyl Cn	Placebo*	Blank	30 µM ranolazine	100 µM ranolazine
C2	145*	313	233	215
C4	0.8*	2.5	1.4	1.6
C6	0.6*	1.1	0.9	1.0
C8	0.19*	0.36	0.27	0.19
C10	0.4*	0.7	0.6	0.7
C12	1.0	0.8	0.7	0.7
C14	0.7	0.7	0.6	0.7
C16	9.0	6.8	7.6	8.0
C16:1	0.9	0.7	0.7	0.7
C16:OH	0.34	0.43	0.46	0.35

In the results, the sponsor states that:

determine the site(s) in fatty acid oxidation affected by ranolazine. However, one possible and already known site of action of the drug could be involved. It is a weak inhibitor of respiratory Complex I (NADH-CoQ oxidoreductase) (SS/023/95), although interestingly, it is more potent in this effect in broken or uncoupled than in coupled mitochondria (which is apparently quite unique (SS/023/95)). Thus, there is potential for its causing a build-up of NADH (which could easily be assayed under the present conditions) and thus inhibiting fatty acid oxidation at the 3-hydroxyacyl CoA dehydrogenase step. However, in the present studies no particular evidence for build-up of 3-hydroxyacyl intermediates was obtained. In a previous study on a patient with <5% normal Complex I activity, a similar degree of fatty acid oxidation flux and reduction in acetyl CoA was observed as at present, but in this case hydroxyacyl intermediates were seen to build up (Watmaugh *et al.*, 1990). The build-up of acyl carnitine intermediates perhaps suggests that the acyl-CoA dehydrogenase (which uses FAD) is a more likely site. The incubation conditions used are likely to cause most of the mitochondria to be coupled, and in this case the effect of ranolazine on Complex I is very weak (k_i >300 μM) (SS/023/95). NADH build-up would also lead to inhibition of flux through pyruvate dehydrogenase and glucose oxidation, yet these are promoted by ranolazine (SS/016/95; SS/018/95; SS/019/95; SS/021/95; SS/022/95). Also, the

Electrophysiology
N.B.- The following are studies specifically identified by the sponsor as crucial to their claims.

CVT303.034-P Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle. Oct. 2000-July 2001. Report date July, 2001.

Isolated left ventricular cells: Whole cell currents were recorded from isolated canine left midmyocardial and epicardial cells at 37°C using conventional whole cell patch clamp techniques. I_{k1}, I_{Ks}, and I_{Kr} were recorded at 37ºC using whole cell voltage clamp techniques. I_{K1} was measured using an external solution containing oubain and nifedipine to block the sodium-potassium current and L-type calcium current (I_{Ca}, L) respectively. I_{Ks} was measured in the presence of E-4031 and nifedipine to block I_{Kr} and I_{Ca}. Ranolazine was applied to the cells at concentrations of 0.1, 0.5, 1.0, 5.0, 10 and 100 μM. I_{Ks} was elicited by depolarization to 40 mM for 3 sec from a holding potential of -50 mV followed by a repolarization step to 0 mV (4.5 sec). The time-dependent tail current elicited by the repolarization was termed I_{Ks}. I_{Kr} was measured as the time-dependent tail current elicited at a potential of -40 mV following a short depolarizing pulse to 30 mV. I_{K1} was recorded during 900 msec of 10 mV voltage steps applied from a holding potential of -40 mV to test potentials ranging from -100 mV to 0 mV, and was characterized as the 5 msec average of the steady state current at the end of the test pulse.

Action potentials were recorded from epicardial and M cell preparations. The effects of ranolazine were determined at concentrations of 1,5,10, 50 and 100 μM, with recordings started 30 minutes after the addition of each concentration of drug. Rate-dependence of ranolazine's effects were determined by recording transmembrane action potentials at BCL of 300, 500, 800, 1000, 2000 and 5000 msec. The data recorded at BCLs of 500 and 2000 msec are presented in the report. Vmax was recorded ±10 and 100 μM of ranolazine at a BCL of 500 msec. Two separate experiments were performed, one using standard $[K+]_0$ of 4 mM and the other with low $[K+]_0$ of 2 mM.

Tissue slices from ventricular epicardial and M region: Tissue slices were isolated from left ventricular epicardial and M regions and allowed to equilibrate in a tissue bath for 4-6 hours while superfused with Tyrode's solution and paced at a basic cycle length (BCL) of 2 Hz using field stimulation.

Left ventricular wedges were placed in Tyrode's solution of either standard $[K+]_0$ of 4 mM or low $[K+]_0$ of 2 mM. Transmembrane action potentials were recorded from epicardial and subendocardial (M) regions using floating microelectrodes. A transmural pseudoECG was recorded along the same axis as the transmembrane recordings. The wedges were allowed to equilibrate for 2 hours while paced at basic cycle lengths of 2000 msec. A constant flow rate was set before ischemia to reach a perfusion pressure of 40-50 mm Hg.

Results:

Ventricular tissue slices:
The prolongation of APD was hypokalemia-dependent in both the epicardial and M-cells. Prolongation was greater at faster rates for the epicardial preparations (use dependent). Biphasic effects were apparent in some M cell preparations, with APD prolonged at low concentrations and shortened at high concentrations.

Wedge Preparations:
At low K+ concentration, ranolazine produced a dose-related increase in QT interval, T_{peak}-T_{end}, action potential duration and transmural dispersion of repolarization. This is shown in the sponsor's table below.

Table 3. Canine Left Ventricular Wedge: 4 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	164 ± 21	209.3 ± 15.76	204.5 ± 13.9	250 ± 13.93	261.1 ± 15.76	34.25 ± 2.56	43 ± 6
1 μM	176.3 ± 12.25	213.8 ± 13.28	203.3 ± 9.621	254.3 ± 9.15	263.5 ± 10.56	34.5 ± 3.202	26.75 ± 8.045
5 μM	176.5 ± 11.85	219 ± 12.12	207.5 ± 8.627	258.3 ± 11.08	274.5 ± 13.73	37.75 ± 4.09	36 ± 2.449
10 μM	170.5 ± 12.03	216.5 ± 13.41	199 ± 9.083	260.3 ± 12.66	277.8 ± 14.99*	39.25 ± 5.54	30.75 ± 10.46
50 μM	159.5 ± 12.82*	218 ± 15.91	187.8 ± 11.21*	257.5 ± 15.47	279.3 ± 17.21*	41.25 ± 8.37	32.5 ± 6.278
100 μM	152.5 ± 14.44*	220.5 ± 18.26	169 ± 10.5*	247.8 ± 15.32	284.5 ± 14.39*	40.5 ± 4.94	23.75 ± 2.689

* $p<0.05$ vs. control $n\leq4$

Table 4.
Canine Left Ventricular Wedge: 2 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	167.3 ± 5.548	220 ± 5.568	195.3 ± 3.283	254.3 ± 0.882	283 ± 2.08	24 ± 12.57	16 ± 9.238
1 μM	173 ± 2	232 ± 5.508	210.7 ± 13.53	280.3 ± 12.72	311 ± 9.5	35 ± 4.70	28.33 ± 11.46
5 μM	183.5 ± 1.5	252.5 ± 10.5	205.7 ± 7.881	289.7 ± 2.848*	319 ± 4.58	33 ± 1.33	15 ± 7
10 μM	190 ± 2*	265.5 ± 16.5	208.3 ± 3.48	305.3 ± 4.978*	329 ± 2.33	36 ± 4.09	23.5 ± 1.5
50 μM	179 ± 1	276.5 ± 18.5*	214.3 ± 6.333	325.5 ± 5.5*	343 ± 2.84	41 ± 6.35	35.5 ± 3.5
100 μM	167.5 ± 0.5	293.5 ± 21.5*	187.7 ± 4.978	345 ± 14.36*	376 ± 4.48	55 ± 1.00	35 ± 11

*$p<0.05$ vs. control $n\leq4$

The effects in the wedge preparations were hypokalemia-dependent. As only 2000 ms BCL was evaluated, use-dependence cannot be assessed.

The effect on I_{Na} was measured by rate of rise of the upstroke of the action potential. Both concentrations of ranolazine that were tested decreased Vmax.

Ranolazine inhibited I_{Kr} (IC_{50} 11.5μM) and I_{Ks} (IC_{50} 13.4μM) in a concentration dependent manner but did not appear to alter I_{K1}.

Torsade de Pointes were not observed to develop spontaneously. Programmed electrical stimulation did not produce TdP under any of the test protocols.

T-wave morphological changes were noted in both tissue slices and wedges: widened, low and notched, especially at low K+.
There was triangulation of the AP and decreased plateau height in both the epicardial and M cells of the tissue slices.

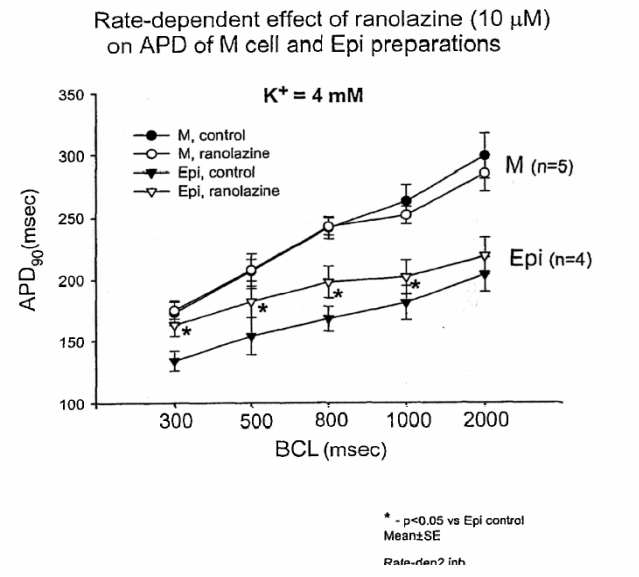
Individual cells, tissue slices and tissue wedges were used in this study. It was not always clear as to which was represented in the results as shown and the reporting was incomplete. Another point that required clarification was the stability of the various test systems over time. This is relevant given the length of the equilibration periods. The duration of exposure to the drugs was not specified for the wedge preparations. It should be noted that evaluations in the presence of

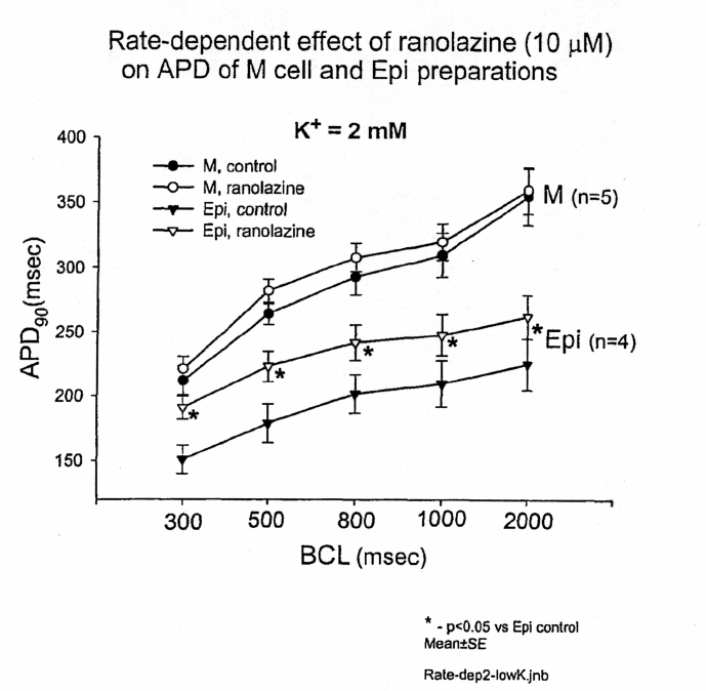
catecholamines have not been made. It should also be noted that ranolazine behaved differently in the tissue slices versus the wedge preps. This is summarized in the reviewer's table below.

preparation	APD50	APD90
Tissue slice	4mM K+: concentration-dependent shortening	4mM K+: concentration and use-dependent shortening
Wedge	4mM K+: decreased (50, 100 μM) 2mMK+: no change	4mM K+: no change More effect on epicardium Than M cells 2mM: prolonged (5-100 μM) more effect on M cells than on epicardium

Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle. Amendment to CVT 303.034P , August 2002.

Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle were given further elaboration in this report, in fact to add the data from isolated myocytes, tissue sections and wedge preparations that were not included in the main report. Ranolazine at 10 μM was tested for its effect on the APD90 at different basic pacing cycle lengths (BCL) of 300, 500, 800, 1000 and 2000 msec in tissue slices of epicardial and M cells. E-4301, an I_{kr} blocker was used for comparison. The superfusing Tyrode solution contained either 2 mM K+ or 4mM K+. At 4 mM K+, and at pacing rates below 1000 msec, there was prolongation of APD90 in the epi cells but not the M cells. In the M cells, at BCL of 1000 and 2000, there was a slight shortening of the APD90. The effect in the epi cells appeared to be greater at lower BCL. The E-4301 caused a marked prolongation of APD90 in the M cells and a minimal change in the epi cells. At 2 mM K+, ranolazine produced a prolongation of APD in both M cells and epicardial cells. The sponsor's graphs are shown below.





Ranolazine's effect in this system appeared to be greater at longer cycle lengths and at low potassium concentrations.

CVT303.036-P Electrophysiologic effects of ranolazine on late I_{Na}, I_{Ca}, I_{to} and I_{Na-Ca} in isolated canine left ventricular myocytes. July 2001-January 2002. Reported January 2002.

Whole cell currents were recorded from isolated canine left ventricular midmyocardial and epicardial cells at 37°C using conventional patch clamp or perforated patch clamp (I_{Na}) to determine concentration-response relationships for the transient outward current (I_{to}), late sodium (late I_{Na}), calcium (I_{Ca}) and sodium-calcium exchange (I_{Na-Ca}) currents. The single

myocytes were obtained following enzymatic dissociation. The effects of ranolazine were determined over the concentration range of 0.5 – 100 μM. Tetrodotoxin was used to block steady state of the late I_{Na} channels. $CdCl_2$ was used to block I_{Ca}. For each ion channel, the sponsor states in the methods that a series of pulses was applied and then the effects measured in the final pulse of the sequence or 4 minutes after addition of each drug concentration. This procedure was repeated for each concentration tested. Data was not provided as to the stability of the test system during this interval.

Results: Ranolazine had minimal effect on $I_{to.}$ 100 μM reduced I_{to} by 16±3% and 17±4% at potentials of 0 and 10 mV respectively ($p<0.001$). Late I_{Na}, I_{Ca} and I_{Na-Ca} were significantly inhibited by ranolazine with IC_{50} values of 21, 296 and 91 μM respectively.
The sponsor compares the effects with those of amiodarone (reduced I_{kr}, I_{ks}, late I_{Na} and I_{Ca}) and proposes this to explain why ranolazine like amiodarone prolongs the QT interval but may not cause torsades de pointes (TdP). However amiodarone does cause TdP in a small percentage of patients. The effect on late I_{Na} is postulated as to perhaps reduce transmural dispersion of repolarization and suppress EAD activity.

CVT303.050-P Use- and voltage-dependent effects of ranolazine on late I_{Na} during action potential voltage clamp.March 2002-August 2002. Reported August 9, 2002
Whole cell currents were recorded from isolated canine left ventricular midmyocardial cells obtained by enzymatic dissociation using the action potential voltage clamp technique. Late I_{Na} was recorded at 37°C. Action potentials elicited at basic cycle lengths of 300 and 2000 ms were used as command waveforms for the voltage clamp. Drug effects during a train of 30 pulses at repetition rates of 300 and 2000 ms were determined over a concentration range of 1 – 50 μM. Late I_{NA} was defined as the tetrodotoxin sensitive current.

Results: Late I_{Na} was evaluated during both the plateau and final repolarization of the action potential clamp. Increasing concentrations of ranolazine caused a decrease in late I_{Na} normalized current with an IC_{50} value of 20.75μM for a BCL of 2000ms, strikingly close to the IC_{50} of 21μM reported in a previous study (CVT303-036P). The IC_{50} was reported as 11.53 μM for a BCL of 300mS which the sponsor proposes as suggestive of use dependence. Another interpretation is that the numbers are the same within normal experimental variability.
According to the sponsor, potency of the block was greatest at plateau potentials and during rapid stimulation. At BCL of 2000 msec, IC_{50} was 20.75 μM with a plateau voltage of –28 mV and 5.86 μM with a plateau voltage of 20 mV. At a 300 ms BCL, half-inhibition of late INa was 5.04 μM during the plateau at a voltage of 13 mV and 11.5 μM during the final repolarization at a voltage of –28 mV. The sponsor's conclusion was a voltage and use dependent inhibition of late I_{Na} by ranolazine. The study would be stronger for the inclusion of positive controls or comparator compounds.

CVT303.042-P Electrophysiological effects of ranolazine in isolated canine purkinje fibers. September 2001- November 2001. Reported January 2002.

Standard microelectrode techniques were used to record transmembrane action potentials from free-running Purkinje fibers isolated from canine right and left ventricles. The preparations were

placed in tissue baths and allowed to equilibrate for ≥30 minutes while superfused with oxygenated Tyrodes solution and paced at a a basic cycle length of 1 Hz. The tissues were exposed to gradually increasing concentrations of ranolazine (1, 5, 10, 50 and 100 μM) at 20-30 minute intervals. The preparations were stimulated at basic cycle lengths of 300, 500, 800, 1000, 2000 and 5000 msec. Data from only the BCLs of 500 and 2000 msec were presented as representative of the relative pacing rates. Two separate sets of experiments were performed using extracellular K+ concentrations ($[K+]_o$) of 3 and 4 mM. The sponsor does not present data indicating the stability of the preparation over time.

Results

At a $[K+]_o$ of 4 mM, ranolazine altered resting membrane potential at concentrations of 50 and 100 μM. At these same concentrations, Overshoot and phase 0 amplitude of the action potential were decreased. This is shown in the reviewer's version of the sponsor's table.

Effects at [K+] =4.0 mM, BCL = 500 msec

	control	Ranolazine in μM				
		1	5	10	50	100
Amplitude	122±5	120±9	124±3	122±7	117±7	106±12*
RMP	-91±1	-90±2	-90±2	-90±3	-89±3	-87±3*
overshoot	32±4	32±7	34±7	32±6	28±7	19±11*

Values are mean ± SD, n=7, * $p<0.05$ compared to control

Lowering $[K+]_o$ to 3 mM did not substantially modify the effects of ranolazine on electrophysiological parameters of Purkinje fibers. Early afterdepolarizations were not reported for any conditions. Graphs of APD versus concentrations of ranolazine show a downward drift with increased concentration. Is this truly a specific effect or due to deterioration of the test system? Stability data or a reference system would be helpful to answer this question. EAD induced by d-sotalol (100 μM) was suppressed by ranolazine at concentrations down to 5 μM.

The next 3 study reports were not identified by the sponsor as critical to the mechanistic argument. However, these reports help to characterize the metabolites.

CVT303.037-P Effects of ranolazine and ranolazine metabolites on the duration of action potential of guinea pig ventricular myocytes. Conducted Nov-Dec 2000, April 2001 and reported March 2002.

Whole-cell patch electrode technique was performed on guinea pig ventricular myocytes. Action potentials were induced by 5-ms depolarizing pulses applied at frequencies of 0.5, 1 or 2 Hz. Values of action potential duration at APD50 and APD90 were measured ±ranolazine (3,10 and 30 μmol/l). Action potentials for ranolazine (10μmol) were also determined in the presence of 5μmol/l quinidine at a frequency of 0.25Hz.

To determine the effect of the selected metabolites, ventricular myocytes were paced at a frequency of 1 Hz. The action potential duration was measured ± one of three metabolites: RAN-2 (RS-94287)(3,10 and 30 μmol/l), CVT2512(RS-88640)(10 μmol/l) and CVT2514(RS-88390)(10μmol/l).

Results: Independent of the pacing frequency, ranolazine caused a dose-related decrease in both APD50 and APD90. The shortening was partially reversible after washout of the drug. At 10 μmol/l ranolazine attenuated the effect of quinidine. The 3 metabolites that were tested had no effect on the duration of action potential elicited at a frequency of 1Hz.

The study would be stronger with the inclusion of positive controls and if ranolazine and the metabolites had been tested according to the same parameters (concentrations and pacing frequencies). If there is a rate-dependent prolongation of action potential duration, the conditions of the present study would not necessarily show the phenomenon. That is, a faster stimulation frequency may be needed. The sponsor does make the point that the decrease in action potential observed in the presence of ranolazine may be attributed to the fact that ranolazine inhibits $I_{Ca(L)}$.

CVT303.040-P Effects of ranolazine, ranolazine enantiomers SAR-103143/SAR-85179, metabolites RS-88390, RS-88640 and RS-94287 and comparators dofetilide and verapamil on HERG and IsK. September 2002.

Concentration-response relationships for dofetilide (1, 10, 100 nM, 1 and 10 μM) and verapamil (1, 3, 10, 100 μM and 1 mM) to block HERG currents were obtained. After baseline I_{HERG} measurements were obtained in the absence of drugs (control), then progressively higher concentrations of dofetilide or verapamil were added to the superfusion solution until complete block was achieved. Steady state responses to both drugs were recorded. Currents from Xenopus oocytes expressing HERG were also recorded under control conditions and in the presence of 10, 30, 100 μM and 1 mM ranolazine. The sponsor did not specify steady state conditions nor was data presented to indicate this. The effects of the S-enantiomer(SAR-103143) and R-enantiomer (SAR-85179) on HERG and IKs was tested under control (no drug) conditions or in the presence of 10μM and 100 μM S- and R-enantiomer. The effects of the named metabolites were determined at concentrations of 0, 10μM and 100μM.

Results: Dofetilide and verapamil inhibited HERG in a concentration-dependent manner with no voltage dependence observed. Ranolazine significantly inhibited HERG currents in a dose and voltage dependent manner. At a concentration of 100μM, ranolazine inhibited HERG by ~50%. In the graphical presentation of results, it was shown that sufficiently high concentrations of ranolazine caused complete I_{HERG} block. Voltage dependence of the block was also observed. The reviewer has summarized this in the table below.

Reviewer's summary of results presented in report text

Ranolazine conc	Isk voltage	Significance
100μM	0-40mV	P<0.05
300μM	-20-40mV	P<0.01,p<0.001
1mM	-30-40mV	P<0.05
3mM	-30-40mV	P<0.01, p<0.001

R- and S- ranolazine also inhibited HERG in a concentration and voltage dependent fashion.

Reviewer's summary of results presented in report text

Ranolazine conc	Isk voltage	Significance

S-ran 10 μM	30, 40 mV	P<0.05
R-ran 10 μM	30, 40mV	Qualitatively but not statistically significant
S-ran 100 μM	-40, -20mV	P<0.05
	-30, 20-40mV	P<0.01
R-ran 100 μM	-20 and 20 mV	P<0.05
	30 and 40 mV	P<0.01

Neither enantiomer had an effect on Isk at the concentrations tested. But overall, 100μM concentrations of either enantiomer inhibited I_{HERG} by 50%, similar to the potency of ranolazine.

Of the metabolites tested, RS-88390 inhibited HERG with approximately the same potency of the parent compound. No effect of the metabolites on Iks was detected.

CVT303.043-P Electrophysiological effects of ranolazine metabolites in myocytes isolated from the canine left ventricle. Conducted July 2001- February 2002. Reported February 2002.

Cells from the epicardial and midmyocardial regions of the left ventricle were used following enzymatic dissociation from the LV free wall. Rapid delayed rectifier and slow delayed rectifier potassium currents were recorded at 37°C using whole cell voltage clamp techniques. I_{kr} was measured as the time-dependent tail current elicited at a potential of –30mVfollowing a 250 ms depolarizing pulse to 30 mV from a holding potential of –50 mV. I_{ks} was measured as the time-dependent tail current elicited at a potential of –30 mV following a 2 sec depolarizing pulse to 40 mV from a holding potential of –50 mV. Metabolites were tested a final concentration of 50 μM. The voltage clamp protocol was repeated 4 times before drug, 4 times during drug (beginning 4 minutes after exposure of the cell to drug) and 4 times after washout of the drug (beginning 4 minutes after initiation of washout). The data for each of 4 runs was averaged. The stability of the preparation over time was not discussed in the report. For I_{kr} experiments, chromanol, a blocker of I_{ks} was added to the recording solutions. For I_{ks} studies, the I_{kr} blocker E-3401 was added to the recording solutions just before the experiment.

Results: Reduction of the I_{kr} tail current was produced with metabolites RS-94287, RS-88390, RS-89961, RS-88681, RS-89983, RS-88772 and RS-88597. Maximum I_{kr} inhibition (51%) was produced by RS-88390. Metabolites CVT-2543 acid (CVT4786?)and RS-89289 produced significant reduction of I_{ks} (39%). The variability of the I_{kr} measurements for CVT2534 acid were disproportionately large compared to the other measurements in this segment of the study: Mean I_{kr} (pA) of 145.3± 61.6 vs RS-88772, 37.8±6.3 or CVT2738 101.5±14.4. The study would be stronger for the inclusion of positive controls.

CVT303.038-P Effects of ranolazine on QT prolongation and arrhythmia induction in anesthetized dog: comparison with sotalol Sept.2002

AV block was induced in mongrel dogs by radio-frequency ablation. Racemic sotalol (n=5)was given iv at a loading dose of 8 mg/kg and a maintenance infusion of 4 mg/kg/hr. Ranolazine (n=5) was given as 0.5 mg/kg intravenous loading dose followed by a first, second and third infusion of 1.0, 3.0 and 15 mg/kg/hr. One dog received ranolazine as a 1.5 mg/kg loading dose followed by infusions of 15 and 30 mg/kg/hr. Twenty minutes (sotalol) or 30 minutes (ranolazine) after starting the maintenance infusion, electrophysiological measurements of right and left ventricular effective refractory period (ERP), QT and QRS were made at basic cycle lengths of 300, 400, 600 and 1000 ms. After the measurements, bolus phenylephrine challenges (10, 20, 30, 40 and 50 μg/kg) were given intravenously and arrhythmias monitored.

Results: All 5 dogs treated with sotalol died from Torsade de Pointes (TdP). The effective refractory period was reported to be prolonged in a reverse use-dependent manner.

One of the 5 dogs treated with ranolazine died during the 30 mg/kg/hr infusion with no electrophysiological measurements made. The sponsor lists the cause of death as pump failure with no further explanation. Ranolazine increased QT at each of the cycle lengths used, but not to the extent of sotalol. TdP was not observed in any of the ranolazine dogs. Runs of ventricular tachycardia were reported, qualified as that which would be expected from phenylephrine alone.

At a basic cycle length of 1000 ms, sotalol increased QT interval from 333±27 to 441±14ms (32% increase). At this cycle length, the maximum increase reported for ranolazine was 348±9 to 384±14 ms (36 msec, 10% increase). The maximum prolongation of QT interval was seen at 3 mg/kg/hr and declined slightly at the higher dose of 15 mg/kg/hr (although still above control levels). Effects on QT are summarized in the reviewer's table below. There is substantially more variability in the controls for the sotalol arm of the study compared to the controls for the ranolazine group. QTc values were not presented.

Summary of Effects of ranolazine and sotalol on QT interval (ms)

Basic cycle length	Mean QT± SE					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran0.5+1	Ran 3	Ran 15
1000	332.7± 77.00	440.93± 76.93**	348.40± 9.07	352.52± 9.05	384.02± 13.9	369.80± 11.6
600	309.85± 73.60	354.67± 74.73**	318.20± 8.58	323.50± 7.74	345.00± 10.04	336.34± 11.43
400	262.73± 74.53	299.14± 73.53**	285.40± 6.02	286.50± 5.76	306.46± 10.38	302.18± 9.33
300	238.40± 74.07	266.40± 74.07*	263.60± 6.61	266.16± 6.36	272.72± 6.09	274.82± 6.48

The drugs both increased the effective refractory period. The baseline between the two groups of dogs differed slightly. This is summarized in the reviewer's table below.

Summary of Effects of ranolazine and sotalol on RV ERP (ms)

Basic cycle length	Mean ERP RV					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran 0.5+1	Ran 3	Ran 15
1000	206.00±8.86	255.50± 9.56**	240.20±9.9	254.00± 9.31*	249.50±6.19	253.16±7.77
600	191.00±7.1	223.50± 9.07**	218.50±8.93	227.50±8.87	224.50±4.83	229.50±6.19
400	174.00±7.85	195.67± 7.53**	194.00±6.83	201.50±6.45	199.66±3.75	206.50±5.79
300	162.00±6.82	181.33± 8.21**	175.00±5.25	182.84±6.67	181.00±2.32	185.00±5.76

Values are Mean ±SE

Summary of Effects of ranolazine and sotalol on LV ERP (ms)

Basic cycle length	Mean ERP LV					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran 0.5+1	Ran 3	Ran 15
1000	252.50±17.5	286.25± 16.25*	252.16± 14.13	259.38± 18.18	265.43± 19.42	260.43± 19.32
600	227.50±12.5	262.50± 27.5*	226.16± 11.29	233.13± 12.43	238.13± 13.25	237.50± 14.11
400	202.50±15	226.25± 21.25	198.50± 9.7	204.38± 11.01	211.45± 9.2	215.00± 10.05
300	182.50±10	201.25± 18.75	180.50± 7.18	185.00± 8.1	189.38± 8.32	196.88± 17.53*

Values are Mean ±SE

Under the conditions of this study, it appears that ranolazine can prolong QT interval and extend the effective refractory period of both ventricles although not to the extent of sotalol. We do not know the blood levels of ranolazine achieved in this study.

Pharmacology summary: The sponsor claims in Item 5, volume 1, p.36, that ranolazine increases pyruvate dehydrogenase activity (PDHa) and inhibits the long-chain and short-chain enoyl-CoA hydratase and carnitine:acylcarnitine translocase. However, there were multiple studies to show that ranolazine binds to several non-target receptors. For example, both enantiomers and the racemate interact with α and β adrenergic receptors as well as 5HT1 receptors. This is summarized in the most recent study, a standard radioligand binding assay. There are further studies showing interaction with opioid receptors and some cardiac calcium channel blocking ability. The parent drug has also been shown to block the I_{kr} channel and to delay repolarization. The properties of the metabolites are incompletely described. For example, receptor binding profiles for the

major metabolites have not been provided and the electrophysiological characterization was minimal. Clinically, the drug has also been shown to prolong the QT interval. The sponsor submitted electrophysiology data that they felt supported their position that ranolazine would not contribute to repolarization abnormalities despite the increased QT interval.

Pharmacology Summary: Electrophysiology

The sponsor presents a series of in vitro studies from the laboratory of Charles Antzelevitch, Ph.D., F.A.C.C., one of the leading experts in cardiac electrophysiology. The studies indicate the ability of ranolazine to interact with cardiac ion channels. As the studies would be stronger for the inclusion of positive control data, either historical or concurrent, to indicate the sensitivity of the models, the Division made specific requests for this material which the sponsor provided in the form of published material and a pre-print of studies conducted in the wedge model using other drugs. While ranolazine did not produce early after depolarizations (EAD) in this model, it should be noted that known arrhythmogenic agents such as erythromycin, d-sotalol and cisapride do not produce EADs or torsade de pointes routinely. The incidence reported for spontaneous occurrence in this model was in the order of 20-30% (Antzelevitch, Sun, Zhang and Yan. J Am Coll Cardiol 1996: 28:1836-48; Diego, Belardinelli and Antzelevitch. Unpublished manuscript "Cisapride-induced transmural dispersion of repolarization and torsade de pointes in the canine left ventricular wedge preparation during epicardial stimulation."Shimizu and Antzelevitch. J Am Coll Cardiol 2000; 35: 778- 786). The series of provided references also suggests that testing compounds in the presence of beta adrenergic stimulation is necessary for complete characterization (J Am Coll Cardiol 2000; 35:778-786). In the wedge studies provided, ranolazine was not tested with catecholamines or some other form of beta adrenergic stimulation. It should be noted that several studies showed that approximately 7 of the major, identified, metabolites have the ability to interact with cardiac ion channels. Several of these metabolites show in vitro potency comparable to the parent drug. More detailed commentary on the electrophysiology in general and the wedge model in particular will be provided in a separate document by John Koerner, Ph.D. In this reviewer's opinion, while the electrophysiology data may potentially be useful as an in vitro elucidation of mechanistic possibilities, it is not possible to extrapolate the information to indicate potential human safety.

Pharmacology Summary: Mechanism of Action

The sponsor presents a series of studies indicating the ability of ranolazine to modulate cardiac energy metabolism. The systems used were isolated mitochondria, various in vitro tissue preparations, perfused hearts and several dog studies. While the data suggest the possibility of direct or indirect effects upon cardiac energy metabolism, the sponsor fails to show how all other potential mechanisms of action were excluded. Should the proposed shift in metabolism from fatty acid oxidation to glucose metabolism actually be the primary mechanism by which ranolazine exerts pharmacologic effects, rather than secondary to some other mechanism, such as calcium channel blockade for example, there are serious concerns surrounding this metabolic shift.

More detailed discussion of proposed mechanism

The sponsor's proposed mechanism of ranolazine's action is a shift from fatty acid oxidation to greater reliance on glucose, primarily through inhibition of carnitine-acylcarnitine translocase and enoyl CoA hydratase.

Ischemia corresponds to partial or total decrease in blood flow to a tissue or organ with loss of oxygen supply to the cells. The hypoxia inhibits oxidative phosphorylation and so ATP decreases while ADP transiently accumulates and is then degraded with an accumulation of phosphate. Anaerobic glycolysis temporarily compensates for the decrease in oxidative phosphorylation but with bi-products of lactate and decreased tissue pH. Persistent ischemia and decreased ATP will lead to failure of the Na+/K+ ATPase which will eventually lead to an increase in Ca^{2+} (Morin et. al. Adv Drug Deliv Rev. 49(2001) 151-174).

After birth, mitochondrial fatty acid β-oxidation becomes the major source of myocardial energy. It has been demonstrated by a number of investigators that defects in the transport, mitochondrial uptake and β-oxidation of long-chain fatty acids causes cardiomyopathy(CM) in infants and children. (Mathur, A. et al. Circulation 1999; 99:1337-1343).

The glucose transporters GLUT1 and GLUT4 have been identified in the myocardium. Insulin and ischemia cause a translocation of these transporters from the intracellular space into the plasma membrane, resulting in an increased capacity for glucose transport. The interstitial glucose concentration is a function of the arterial glucose concentration and blood flow, thus interstitial glucose levels and the transmembrane glucose gradient are decreased during ischemia and increased by hyperglycemia (Stanley, W.C. Cardiovascular Research 33(1997):243-257.). It would thus seem paradoxical that during angina, when glucose is in decreased supply, to shift metabolism to utilize more glucose.

It has already been demonstrated that myocardial ischemia or increased cardiac work will cause a fall in glycogen concentration. Myocardial substrate metabolism during ischemia is dependent upon the severity of ischemia. A decrease in flow of 20-60% causes a decreased myocardial oxygen consumption (~10-50%), a transient increased dependence on anaerobic glycolysis, reduced rate of FFA oxidation and somewhat more severe contractile dysfunction (ibid.). However, the primary oxidative fuel during mild to moderate ischemia is fatty acids. More severe reductions in flow (>70%) result in greater rates of lactate accumulation and glycogen breakdown. When there is complete elimination of flow, there is total dependence on anaerobic metabolism with glycogen as the sole glycolytic substrate as there is no blood flow to deliver glucose to the tissues.

Carnitine-acylcarnitine translocase(CT) is an inner mitochondrial membrane protein that allows transfer of carnitine and its esters across the inner mitochondrial membrane. CT deficiency is characterized by 2 syndromes, the milder of which includes muscle weakness and hypoketotic hypoglycemia. The more severe syndrome includes development of cardiomyopathy. (Antozzi,

C. and Zeviani, M. Cardiovascular Research, 35 (1997): 184-199). Arrhythmias also seem to be associated with inhibition of carnitine-acylcarnitine shuttling to the mitochondria. (Bonnet D., et al. Circulation. 1999;100:2248-2253.)

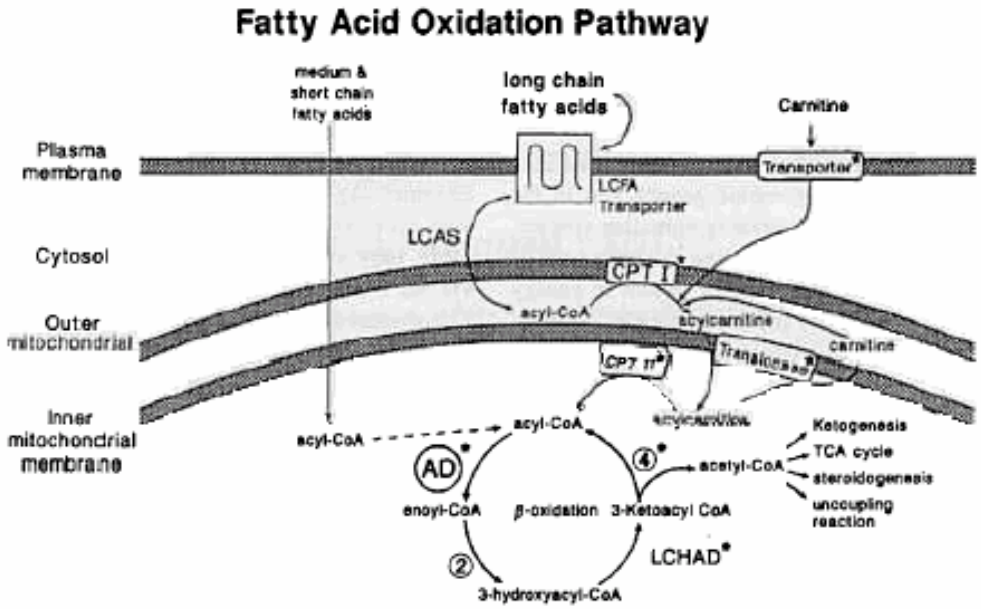


Figure 1. The ß-oxidation pathway of fatty acids and specific defects causing cardiomyopathy. The pathways of fatty acid oxidation and cellular carnitine metabolism are shown. Defects known to cause cardiomyopathy (indicated by the asterisks) include abnormalities in carnitine and acylcarnitine transport-carnitine transport defect, carnitine palmitoyltransferase II (CPT II) deficiency, and carnitine-acylcarnitine translocase (translocase) deficiency - and errors in steps 1 and 3 of mitochondrial ß-oxidation, long-chain and medium-chain acyl-coenzyme A dehydrogenase (AD) deficiencies and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency, respectively. LCFA, long-chain fatty acid; LCAS, long-chain acyl-CoA synthetase.

Thus, the proposed mechanism of action for ranolazine appears to involve shifting to glucose metabolism at a time when glucose is in short supply and also to accumulate the cardiotoxic intermediates of fatty acid metabolism.

The apparent paradox in the sponsor's proposed mechanism of action is succinctly stated in the work by V.G. Davila-Roman et al "Altered myocardial fatty acid and glucose metabolism in idiopathic cardiomyopathy." JACC 2002; 40:271-277. This is shown below in Davila-Roman's own words.

Alterations in myocardial substrate metabolism have been implicated in the pathogenesis of contractile dysfunction and heart failure (HF) (1–3). Animal models of HF have shown that in the progression from cardiac hypertrophy to ventricular dysfunction, the expression of genes encoding for mitochondrial fatty acid beta-oxidation (FAO) enzymes is coordinately decreased, resulting in a shift in myocardial metabolism that recapitulates the fetal heart gene program, with glucose instead of fatty acids becoming the primary energy substrate (4–8).

The reactivation of the metabolic fetal gene program may have detrimental consequences on myocardial contractile function. The downregulation of mitochondrial FAO enzymes is associated with increased myocardial utilization of oxygen-sparing glycolytic pathways for the production of high-energy phosphates (4). Although this allows for reduced oxygen demands in the hypertrophied and failing heart, the reliance of the myocardium on glucose may produce a relatively energy-deficient state that over a long time may result in decreased contractile performance (1–3). Alternatively, the inability to metabolize fatty acids in the presence of excess availability may be associated with accumulation of nonoxidized toxic fatty acid derivatives, resulting in lipotoxicity and HF (9). This hypothesis is supported by the development of myocardial hypertrophy, HF and sudden cardiac death in children with genetic defects in myocardial FAO enzymes (10–12). Furthermore, myocardial FAO enzyme expression is downregulated in humans with dilated cardiomyopathy, suggesting that a gene regulatory program is responsible for the alterations in myocardial energy substrate utilization (13).

Animal studies have provided significant insight into the metabolic alterations that occur in HF; however, studies in

It may be argued that in the naturally occurring deficiencies in fatty acid oxidizing enzymes the degree of deficiency is greater than what would occur in a pharmacologically-induced situation. However, some other considerations remain:

1. Those affected by CAT deficiency show signs generally within the first day or two of life. How long will it take for a lesser degree of pharmacologically-induced deficiency to become apparent? That is, to produce signs of arrhythmia from the build-up of arrhythmogenic intermediates or from heart failure?
2. Why is a pharmacologically induced deficiency less deleterious and more beneficial than the natural deficiency?

The pharmacology studies presented to support the proposed mechanism of action made little use of comparator compounds to get some idea of the sensitivity of the assay systems. For example, Morin et al (Ibid.) reviewed drugs that have been shown to modulate mitochondrial metabolism. Drugs such as diazoxide, amiodarone, carvedilol, gingko biloba, propofol, cyclosporin A, clonazepam and diltiazem can all be shown to influence different enzymes of fatty acid oxidation. However, it can be argued that this is not the accepted therapeutic mechanism of action for any of these drugs.

The sponsor has not presented studies to exclude contribution of other mechanisms such as binding to opioid receptors, calcium channel effects or alpha and beta adrenergic effects. Both opioids and calcium channel blockers may decrease cardiac oxygen consumption, left ventricular end diastolic pressure and cardiac work. While the parent drug shows low to moderate binding to calcium channels, opioid receptors and alpha and beta adrenergic receptors, there is no information apparent as to the affinity of any of the major metabolites for these receptors and channels.

There is an assay that is used clinically to identify those who have an inherited or congenital deficiency in the carnitine:acylcarnitine translocase (Brivet et al. “Rapid diagnosis of long chain and medium chain fatty acid oxidation disorders using lymphocytes.” Ann Clin Biochem 1995;32:154-159; Saudubra et al. “Recognition and management of fatty acid oxidation defects: A series of 107 patients.” J. Inher.Metab Dis 22(1999)488-502.; Brivet et al. “Defects in activation and transport of fatty acids.” J. Inher. Metab Dis.22 (1999)428-441).Why did the sponsor not use this assay to generate data from the species of interest, the human, or at least, human tissue samples, to support the proposed mechanism?

Pharmacology conclusions: The sponsor was given a specific request by telephone to identify the studies that support the proposed mechanism of action and to show how other possible mechanisms were discounted. The sponsor presents numerous studies indicating that ranolazine may exert pharmacological effects upon the metabolism of isolated mitochondria, various organ preparations and some dog models. No evidence is presented to show how other possible mechanisms of action (the “non-target” receptors to which ranolazine and the major metabolites bind) were eliminated from consideration. The electrophysiology data indicates the potential for ranolazine to interact with cardiac ion channels and may be useful in the in vitro characterization of mechanistic questions. A number of the major metabolites were also shown to interact with cardiac ion channels

equipotent to parent drug. No extrapolations to human safety may be made from this electrophysiology data.

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II. SAFETY PHARMACOLOGY:

Neurological effects:

The effect of RS-43285 on motor coordination in the rat as determined using the accelerating rotarod AT3377,Conducted June 1984, reported April 1985. vol. 4, p. 76

Sprague-Dawley rats (Charles River, UK) of unspecified sex were used. The methods are not entirely clear, but it appears that rats received a single oral dose of 10 mg/kg of RS-43285 and one hour later were observed on an accelerating rotarod apparatus. Length of time each rat remained on the rod was determined. Eight rats per group were used. Clonidine and vehicle controls were used for comparison. Clonidine-treated rats stayed on the rod for approximately 25 seconds on average compared to 75 seconds on average for the vehicle controls and approximately 70 seconds for the ranolazine-treated rats. The study is inadequate in that only one dose of drug was tested. There is no way of assessing the existence or absence of a dose-response effect. In light of what has been observed in essentially every toxicology study in each species and has also been reported in various other pharmacokinetic and pharmacology studies, the reviewer disagrees with the sponsor's conclusion that ranolazine "is unlikely to produce overt CNS sedation or stimulation"

The effect of RS-43285 on clonidine-induced hypoactivity in rats. AT3248, April –May 1983. Reported December 1984.

Ligand binding studies had shown that ranolazine possesses some affinity for the $\alpha 2$-adrenoceptor in rat cerebral cortex. This study examined the effect of single oral doses of 0

(water), 10, 30 or 100 mg/kg ranolazine and possible central α2-adrenoceptor antagonist activity in conscious rats as demonstrated by a reversal of the effect of α2-adrenoceptor agonist clonidine (0.3 mg/kg) on locomotor activity. Group size was not disclosed.

Results were expressed in activity units without explanation of how activity was assessed.

treatment	Activity units
Control (untreated)	1000
clonidine	200-300
Ranolazine + clonidine	
ranolazine	447 (n=1)

There is a problem with the results. In the text, the sponsor states that the control animals showed 1000 units of activity. However, in the graphical presentation of the data, the control animals show much less (≤300 units of activity). If one gives any weight to the results, it must be considered that ranolazine itself appears to have a sedative effect in this model.

Neuropharmacological Activity AT3829,vol 4, p152.
Overt Behavior: Groups of 3 male Sim:(ICR)fBR were given intraperitoneal doses from 10-300 mg/kg of ranolazine. Exact doses were not specified. The sponsor reports normal behavior at 10 mg/kg. Crouching posture was noted at 30 mg/kg in one (1/3 or 30%) animal. At 100 mg/kg, signs reported include decreased spontaneous locomotion, wobbly gait, decreased induced activity, decreased grip strength, loss of orientation, ataxia, loss of righting reflex, decreased muscle tone, decreased muscle temperature and mydriasis. Acute mortality occurred at 300 mg/kg for 3/3 mice. Cause was not determined. The sponsor states that 100 mg/kg i.p. "…was a central nervous system depressant."

Induced aroused and unaroused loss of the righting reflex in the mouse: Five groups of ten male Sim:(ICR)fBR mice were given intraperitoneal doses of 100, 110, 120, 160 and 250 mg/kg. At periodic intervals after dosing the mice were placed on their backs. If mice were unable to right within 30 seconds they were regarded as having lost the righting ability while in the unaroused state (ULRR). The mice were then aroused by vigorously rolling in a person's hands for 10 seconds and again placed on their backs. If they were now unable to right, they were considered to have lost their righting ability in the aroused state (ALRR) The ratio of ED_{50} (mg/kg, ip) for unaroused and aroused loss of righting was calculated and compared to data for known central nervous system depressants. This is summarized in the reviewer's table below.

Compound	ED_{50} (mg/kg, i.p.) and 95% confidence limits		AED_{50}/UED_{50}
	Unaroused	aroused	
RS-43285	120 (112-128)	174 (160-190)	1.45
phenobarbital	112(99-121)	142(136-150)	1.3
glutethimide	105(91-115)	115(109-122)	1.1
promazine	65(55-73)	91(81-103)	1.4

The sponsor notes that the ratio of aroused:unaroused ED50s was a value comparable to that of phenobarbital and meprobamate, suggesting general CNS depressant activity.

Induction of neurological deficiency (Neurological and skeletal muscle coordination and function): Groups of 10 male Hla: (ICR)BR mice were dosed intraperitoneally with either water or doses of ranolazine from 10-100 mg/kg. Fifteen minutes after dosing, the mice were observed for their ability to remain on a suspended wire for 10 seconds. Immediately after this the mice were subjected to an electroshock test. The drug was effective in inducing a neurologic deficit with an ED_{50} of 64 (53-70) mg/kg. Sedation was reported for higher dose levels. Mean time on the wire was decreased compared to the controls at all doses and showed a dose-related response. Therefore, neurologic effects were apparent even at the lowest dose of 10 mg/kg. Sponsor's results are shown below.

Summary of Test for Induction of Neurological Deficit

compound	Dose mg/kg	#unimpaired/#tested	Mean time on wire sec±SEM
water		10/10	30±0
RS-43285	10	9/10	27.5±2.5
	30	10/10	21.2±2.4
	60	6/10	11.5±1.4
	80	2/10	4.9±1.3
	90	0/10	1.8±0.8
	100	0/10	2.2±1.1

No NOEL was found for the induction of a neurological deficit.

Effect on Hexobarbital-induced sleep time: Four groups of 10 male Sim: (ICR)fBR mice were used. The mice were treated with water or an aqueous solution of ranolazine at doses of 1, 10 and 50 mg/kg i.p. Fifteen minutes after dosing, 100 mg/kg hexobarbital was given intraperitoneally. Mice were observed for onset and duration of loss of the righting reflex (sleep). Onset of sleep was not reported. Duration of hexobarbital-induced sleep was significantly ($p = 0.03$) increased at the highest dose of 50 mg/kg: 112±11 vs 74± 12 min for the control.

Effect on the electroencephalogram, reticular formation multiple unit activity and sleep of cats: The three cats used in the study were from a colony of cats having electrodes implanted at various specified loci in the brain. Ranolazine was given at 10 or 30 mg/kg. Each cat received both doses with at least 2 weeks between doses. Baseline recordings of EEG, RFMUA(a signal characteristic of the sleep stages in the cat) and EMG were obtained for 48 hours prior to dosing. No observations were made for the first 24 hours to eliminate the first night effect. EEG and behavior were observed for 1 hour. Each cat receiving the HD showed signs of salivation, retching and emesis. The sponsor reported that there were no effects on the parameters measured. However, there was a dose-related increase in REM sleep and REM latency as shown in the reviewer's summary of the sponsor's data below:

Summary of effects on Cat REM sleep (N=3)

	Mean percent ±SE of 24 hour			
	Day -1	Day 1	Day 2	Rem latency min± S.E.
Historical control	14.1±0.3	12.2±0.8	12.9±1.1	90±33

Ran 10 mg/kg	13.8±2.3	14.3±1.4	13.6±1.3	92±21
Ran 30 mg/kg	16.5,17.4*	12.5±1.7	14.3±1.2	107±10

*data on one cat was lost due to recorder failure

While the differences are not significant, there does appear to be a mild dose-related effect upon the duration of REM sleep.

Hot Plate Analgesia Effects: Groups of ten male Hla: (ICR) BR mice were tested 3X at 30 minute intervals prior to dosing to establish a baseline. Water or ranolazine at doses within the range of 1 – 100 mg/kg was administered intraperitoneally. The mice were then tested three more times at 30, 60 and 90 minutes after receiving the drug. Drug efficacy was defined as doubling of each individual mouse's average control response latency. Ptosis and sedation were reported for the HD. Latency relative to the control was not apparently affected. There was therefore no apparent effect on analgesia.

Stress-Induced Hyperphagia in rats: Groups of eight male Hla: (SD)BR rats received either water or ranolazine at 1,10 or 100 mg/kg p.o. Thirty minutes later, the rats were restrained and allowed access to sweetened condensed milk. The volume of milk consumed was measured. There was a dose-related increase in the amount of milk consumed in the treated animals as shown in the reviewer's table below.

Summary of milk volume consumed

treatment	Volume of milk consumed ml±sem
Water	4.7±1.0
Ranolazine 1mg/kg	5.0±1.0
10	5.3±1.1
100	5.9±1.2

If the premise of the study is that stress causes an increase in milk consumption, then it would appear that increased doses of drug caused increased stress.

Anti-convulsant effect using electrically-induced seizures: Groups of 10 Hla: (ICR) BR mice received either water or drug intraperitoneally. Doses used were 10, 30, 60, 80, 90 and 100 mg/kg. At 10 and 30 mg/kg there was increased mortality (80% and 90% respectively compared to 40% for the control group) as a result of the seizures. At 80 and 90 mg/kg the tonic extensor seizures were incomplete. The animals were also observed to be sedated at these doses. At 100 mg/kg the seizures were absent (0/10). The animals also appeared to be sedated.
Low doses of ranolazine appeared to increase the lethality of seizures. The high doses that caused signs of sedation decreased the incidence of induced seizures.

Antagonism of apomorphine-induced climbing: Two studies were conducted using groups of 8 male Sim: (ICR)fBR mice. The mice were treated intraperitoneally with vehicle or ranolazine 15 minutes prior to dosing with 3 mg/kg apomorphine subcutaneously. Six minutes later and every

30 seconds for 15 minutes, the mice were observed for climbing behavior. The initial study used doses of 0.3-30 mg/kg. The LD-treated mice showed significantly less climbing behavior than the controls. Therefore the doses were decreased in the subsequent study to 0.03- 1.0 mg/kg. There were no significant differences in the second study.

Oxotremorine-induced tremors (central anticholinergic activity): Four groups of 10 male Sim: (ICR)fBR mice were treated with water or ranolazine at 1, 10 or 50 mg/kg i.p. Fifteen minutes later, oxotremorine 2 mg/kg was given intraperitoneally. The mice were observed for tremors at 5, 10,15 and 30 minutes after oxotremorine administration. There was no apparent effect upon numbers of tremors and no other signs were reported.

Effects of ranolazine in in vitro neurotoxicity models. AT7011 July 1994-April 1995. Reported June 1995.

Ranolazine(1-100μM) was tested using cultured rat hippocampal neurones against 1) glutamate-induced cell death and 2) glutamate-induced intracellular Ca^{2+} transients. All further experimental details were referenced to 2 papers.
Results: There was a statement that ranolazine did not alter, prevent or protect cultured rat hipocampal cells from 10μM glutamate. No data was presented.

Effects of RS-43285 on the electroretinogram of guinea-pig retina. AT4944 Jan 1989- Feb 1989. Reported August 1989.

Female guinea pigs of unspecified breed (albino Duncan Hartleys?) were euthanized, the eyes collected and the retina isolated. After a 1 hour equilibration period control ERG's were recorded and the tissue then made anoxic by bubbling the superfusing solution with 100% nitrogen. ERG's were again recorded 2 and 4 minutes after beginning anoxic conditions. After 4 minutes of anoxia, the superfusing solution was bubbled with 100% oxygen and a recovery ERG recorded. The protocol was repeated after 30 minutes to obtain a second control. Fifteen minutes later the anoxia procedure was repeated. When recovery from anoxia was observed, the ranolazine was removed from the superfusing solution and "30 minutes left before obtaining a final control response from the retina to anoxia." Some additional control preparations were treated as described above with the exception that the RS-43285 was not added to the perfusing solution on the third anoxic challenge.

Results: There were no significant differences reported between the anoxia-induced reduction in ERG amplitude ± ranolazine (10^{-5}M) at either time point. Given that there was no comparator compound and only one concentration of ranolazine was tested, limited information is derived from the study.

Cardiovascular effects:

Hemodynamic effects of intravenous infusions of RS-43285 in pentobarbitone-anesthetised dogs. AT3300, vol 3, p.325. June 1984-Sept 1984. Reported January 1985.

Mongrel dogs were infused with cumulatively increasing doses of 1, 10, 100 and 1000µg/kg/min with each dose infused for 15 minutes. The results were compared to those obtained in control animals given saline (1 ml/min for 1 hour). Flow probes were installed in the LAD and aortic root. Pressure transducers were used for pulmonary arterial flow and left ventricular pressure. A lead II ECG was recorded.

Results: The dogs infused with saline showed no apparent changes in cardiovascular function. The dogs receiving the 2 lowest doses of ranolazine showed approximately 22% increase in coronary blood flow. LVEDP was approximately doubled at these two doses also. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) showed dose-related decreases. The sponsor's results are shown here.

There were dose-related decreases in ABF (aortic mean blood flow), SV, MAP, SAP, DAP, HR,

HEMODYNAMIC EFFECTS OF RS-43285 IN PENTOBARBITONE-ANESTHETISED DOGS (N = 4)

Parameter	Initial Baseline value	Values during RS-43285 infusion at various doses for 15 min µg/kg/min i.v.			
		1	10	100	1000
CBF	45.0 ± 4.2 ml/min	54.5 ± 8.0	56.0 ± 13.0	47.0 ± 3.0	42.5 ± 8.0
CVR	2.14± 0.5 mmHg/ml/min	1.6 ± 0.2	1.9 ± 0.4	1.5 ± 0.2	1.3 ± 0.3
ABF	1.3 ± 0.2 l/min	1.2 ± 0.2	1.3 ± 0.2	0.7 ± 0.1*	0.5 ± 0.1*
TPR	77.7 ± 13.0 mmHg/l/min	82.0 ± 9.0	74.0 ± 15.0	103.0 ± 6.0	122.0 ± 19.0
SV	8.2 ± 1.8 ml/beat	7.5 ± 2.0	8.3 ± 2.0	4.6 ± 0.5	3.2 ± 0.6*
MAP	94.9 ± 2.6 mmHg	95.6 ± 4.0	88.0 ± 7.0	71.0 ± 14.0	51.0 ± 13.0*
SAP	115.0 ± 2.8 mmHg	112.0 ± 5.0	107.0 ± 9.0	86.0 ± 17.0	66.0 ± 14.0*
DAP	83.6 ± 2.0 mmHg	86.0 ± 5.0	77.0 ± 6.0	62.0 ± 13.0	42.0 ± 12.0*
HR	167.0 ± 12.0 beat/min	170.0 ± 11.0	168.0 ± 12.0	147.0 ± 22.0	134.0 ± 14.0*
LVSP	125.0 ± 18.0 mmHg	127.0 ± 15.0	124.0 ± 17.0	93.0 ± 35.0	77.0 ± 26.0*
LVEDP	4.3 ± 2.4 mmHg	7.0 ± 2.8	8.0 ± 3.0	3.5 ± 2.0	8.0 ± 4.0
+dp/dt	3893.0 ± 241.0 mmHg/sec	3962.0 ± 219.0	3928.0 ± 157.0	2313.0 ± 793.0	1672.0 ± 525.0*
-dp/dt	5381.0 ± 772.0 mmHg/sec	5451.0 ± 699.0	4831.0 ± 570.0	3871.0 ±1449.0	2572.0 ± 946.0*
dp/dt/P	33.75± 2.6 sec^{-1}	34.5 ± 2.0	37.0 ± 4.0	21.4 ± 7.0	21.0 ± 7.0
LVMW	2.2 ± 0.2 kg.m/min	2.2 ± 0.3	2.3 ± 0.3	1.2 ± 0.6*	0.3 ± 0.6*
PAP	10.5 ± 1.5 mmHg	11.0 ± 1.7	10.1 ± 1.3	9.6 ± 1.2	10.0 ± 2.0

* denotes difference with respect to initial value significant $p < 0.05$ by analysis of variance or t-test.

LVSP, +dp/dt(left ventricular contractility), dp/dt/p (left ventricular contractility) and LVMW (LV minute work). No ECG data was presented.

Increasing doses of ranolazine caused loss of cardiovascular function manifested as decreased cardiac output, contractile force and left ventricular systolic pressure. Left ventricular minute work was decreased while total peripheral resistance was increased. The sponsor notes that the 2 higher doses were equivalent to total doses of 25 mg and 250 mg for a 15 kg dog.

25 mg/15kg= 1.67 mg/kg ÷ 1.8 = 0.93 mg/kg Human equivalent dose (HED)
250 mg/15 kg = 16.67 mg/kg ÷1.8= 9.26 mg/kg HED

Human Cmax: 5290 ng/ml

A 70 kg human receiving the maximum dose of 1000 mg ranolazine receives a dose of 14.28mg/kg. Therefore, the dogs showing depression of cardiac function in the above study are not even receiving exposure equivalent to the human therapeutic levels.

Effect on norbormide-induced sudden death in rats: Norbormide at 50-200µg/kg produces general vasoconstriction, coronary vasoconstriction, arrythmias, respiratory depression, hypotension and cardiac arrest in rats. An unspecified number of male Sim: (SD)fBR rats per group were anesthetized, vessels cannulated and maintained on artificial respiration. The rats received drug intravenously 30 seconds – 2 minutes before 125µg/rat of norbormide was given i.v. Ranolazine had no effect on survival at doses of 1,3,5 or 20 mg/kg. At 10 mg/kg 3 animals survived (0 survived in all other groups including the controls). There was no dose-related effect. The results are most likely accidental or spurious.

Hemodynamics in the conscious dog: Two instrumented female dogs trained to slings were used. Baseline readings were made then isoproterenol was continuously infused in step-wise fashion using 3 doses (0.02, 0.07 and 0.21 µg/kg/min) for 5 minutes at each dose. Monitoring was done at the end of each 5 minutes. The animal was allowed to recover to pre-isoproterenol levels. Ranolazine or propylene glycol was given in a gelatin capsule. Each dog was observed for 3 hours post-dose and readings made at 15 minute intervals. Doses of ranolazine used were 200 µg/kg, 1 mg/kg and 5 mg/kg. Each dose was given to a single dog on a separate day. The HD was studied twice while the LD and MD were studied only once. The results, for n=1, do indicate drug effects upon cardiovascular parameters. However, the study is underpowered and inadequate. In the multi-study summary that accompanies the set of studies in which this one was included, the sponsor mentioned that the positive inotropic and chronotropic effects of challenges with epinephrine, norepinephrine and isoproterenol were mildly inhibited, but not the blood pressure decrease elicited by isproterenol, suggesting β-adrenergic blocking properties (p.303). The results were determined in a single dog (n=1, p.329).

Hemodynamics in an anesthetized dog: One dog was anesthetized, a midsternal thoracotomy performed and strain gauges used to determine contractile force. Bilateral vagotomy was also performed. Ranolazine was given intravenously at doses of 0.316, 1.0 and 3.16 mg/kg at intervals of approximately 1 hour. Challenge drugs were administered at 10-15 minute intervals before and after each dose of RS-43285. The drugs used were epinephrine, norepinephrine, isoproterenol, angiotensin, acetylcholine, histamine and bilateral carotid occlusion. The sponsor notes that due to time constraints these were not given after each dose of ranolazine. The only numerical data presented was 2 tables, titled "Percent inhibition of heart rate responses following intravenous administration of RS-43285 to an anesthetized dog" and "Percent inhibition of myocardial contractile force responses following intravenous administration of RS-43285 to an anesthetized dog". Only epinephrine, norepinephrine and isoproterenol results were shown. The sponsor states that ranolazine exhibited some "mild cardiac β-adrenergic blocking properties against autonomic nervous system changes." Given the n of 1 and the lack of reported results, the study is inconclusive.

Pulmonary effects:

Respiration in the anaesthetized dog AT3386, vol 4, p.113

Female beagles (E.G. Crowley, Malvern, Worcs.) were anesthetized with sodium pentobarbitone and maintained with this. The dogs were intubated and body temperature maintained. Continuous recording of respiratory rate, tidal volume and flow rate was made. Blood gases were monitored as well as mean arterial blood pressure. The test article was dissolved in saline and administered intravenously giving cumulative doses of 15, 50, 200, 500, 1500, 3000, 7000 and 15000 µg/kg. Blood samples were taken 10 minutes after each dose then measurements recorded for 2 minutes. A dosing-recording cycle of 13 minutes was thus established. The sponsor stated that it was not necessary to show standard error bars as there were no significant differences and thus the error bars were not shown in any of the figures. No positive control data was shown. Results are summarized in the reviewer's table below.

Summary of respiratory parameters (N=3): values approximate

parameter	control	Drug-treated
Femoral venous pH	7.34	50-1500 µg/kg: 7.36-7.37; 3000-15000µg/kg:7.34-7.32
Femoral arterial pH	7.34	All drug-treated groups: 7.36-7.38
Femoral arterial pO_2 (no units)	~72	≥50 µg/kg : 75-80
Femoral venous pO_2 (no units)	50	50-7000 µg/kg: 50-46
Femoral arterial pCO_2(no units)	40	All drug-treated groups: 36-32
Femoral venous pCO_2(no units)	41	All drug-treated groups: 39-33
Expiratory flow rate (ml/sec)	110	15-200µg/kg: 11-115; 500-15000µg/kg: 105-95
Tidal volume(ml)	109	All drug-treated groups: 115-120
Respiratory rate(breaths /min)	15	No apparent effect
Minute flow (ml/min)	1540	200-3000µg/kg: 1700-1800 ml/min
Mean arterial blood pressure(mm Hg)	127	Began falling >500 g/kg. At 15000 µg/kg was 116 mm Hg.

The values were obtained from graphical presentations of the data and are therefore approximate.

The system shows a great deal of variability. A positive control, either historical or concurrent, would strengthen the study.

Renal effects: no studies found

Gastrointestinal effects:

The effect of RS-43285 on gastric secretion in the isolated perfused stomach of the mouse. AT3364 Vol 4, p.60.

Male CD-1 mice were killed by cervical dislocation, the stomachs isolated, cannulated, washed and placed in an organ bath. RS-43285 was added to the serosal solution in a cumulative fashion, with a 15 minute equilibration allowed at each concentration. The pH of the perfusate was determined at the end of each 15 minute period. The concentrations of RS-43285 used were

from $1x10^{-8}$ to $1x\ 10^{-4}$ M. It does not appear that control samples were included in the study design. No experimental results were shown. The sponsor stated that the concentrations tested were without effect on the pH of the perfusate. The study is inconclusive.

The effects of RS-43285 on the intestinal transit of radiochromium (^{51}Cr) marker in vivo AT3365 Vol 4., p68. Male CD-1 mice were orally treated with either RS-43285 (80 mg/kg) or saline vehicle. Thirty minutes later 0.2 ml of radiochromium (0.5μCi $Na^{51}CrO_4$ in saline) was orally instilled into the stomach. Thirty minutes after chromium administration the animals were killed by cervical dislocation. The small intestine was removed and divided into 10 equal segments. The radioactivity in each segment was assessed by gamma counting. Differences in gastrointestinal transit were assessed by calculation of the geometric center (GC) using the equation GC= sum of (fraction of ^{51}Cr per segment x segment number). No results were presented except for the sponsor's statement that GC for the vehicle was 6.38±0.34 and for the drug was 6.05±0.66. Use of positive controls, either historical or concurrent would strengthen the study.

Abuse liability: no studies found

Other:

Safety pharmacology summary: Safety pharmacology data was presented for the cardiovascular, gastrointestinal, neurological and pulmonary systems.
The cardiovascular safety study showed that cumulatively increasing intravenous doses of 1, 10, 100 and 1000 μg/kg/min , with each dose infused over 15 minutes, caused a profound deterioration in cardiovascular function at 100 and 1000 μg/kg/min manifested as decreased cardiac output, contractile force and left ventricular systolic pressure. Left ventricular minute work was decreased while total peripheral resistance was increased. There were dose-related decreases in ABF (aortic mean blood flow), SV, MAP, SAP, DAP, HR, LVSP, +dp/dt(left ventricular contractility), dp/dt/p (left ventricular contractility) and LVMW (LV minute work). No ECG data was presented. The sponsor was contacted by telephone 5/16/03 and asked to provide the ECG data.

There was no plasma drug concentration data. However, the sponsor notes that the 2 higher doses were equivalent to total doses of 25 mg and 250 mg for a 15 kg dog.

250 mg/750 ml = 0.33 mg/ml
human Cmax 5290 ng/ml

25 mg/15kg= 1.67 mg/kg ÷ 1.8 = 0.93 mg/kg Human equivalent dose (HED)
250 mg/15 kg = 16.67 mg/kg ÷1.8= 9.26 mg/kg HED

A 70 kg human receiving the maximum dose of 1000 mg ranolazine receives a dose of 14.28mg/kg. Therefore, the dogs showing depression of cardiac function in the above study are not even receiving exposure equivalent to the human therapeutic levels.

The neurologic effects evaluation included: motor coordination (accelerating rotarod), overt signs, induced aroused and unaroused loss of righting reflex, induction of neurologic deficiency, effect on hexobarbital sleep time, effect on the electroencephalogram and reticular formation multiple unit activity and sleep in cats, hot plate analgesia effect, stress-induced hyperphagia, anti-convulsant effects, antagonism of apomorphine-induced climbing and oxotremorine-induced tremors.

A point that emerged in the overt behavior, induced aroused and unaroused loss of righting reflex, induction of neurologic deficits, hot plate analgesia effects and anti-convulsant effects was that of sedation. The sponsor noted in several of these studies that central sedation appeared to be a characteristic of ranolazine. The sponsor compared the AED_{50}/UED_{50} (aroused/ unaroused) for ranolazine to that of phenobarbital, glutethimide and promazine. Mydriasis was reported in the CNS overt signs study at a dose of 100 mg/kg in addition to decreased activity, ataxia, decreased grip strength, loss of orientation, loss of righting reflex and decreased temperature and muscle tone. In the neurologic deficits study, deficits were elicited at every dose tested (no NOEL identified) with an ED_{50} of 64 mg/kg (range of 53-70 mg/kg). Sedation was reported for doses ≥80 mg/kg. This is supported by the hexobarbital sleep study in which 50 mg/kg, the highest dose tested, significantly (p=0.03) prolonged the sleeping time (a dose not associated with sedation in these studies). Although sedation was noted in the hot plate analgesia test, there was no apparent effect on analgesia.

Stress-induced hyperphagia yielded interesting results. Treatment with ranolazine produced dose-related increases in the volume of milk consumed after restraint-stress.

The antagonism of apomorphine-induced climbing produced effects where ranolazine-treated mice showed significantly less climbing behavior than the controls. When the doses were decreased in a subsequent study to 0.03 –1 mg/kg (clinically irrelevant exposure levels) there were no significant differences. Note: apomorphine is a potent dopaminergic stimulant in mice. The results showing antagonism of this effect may have been due to dopaminergic antagonism or general sedation (although that was not reported). The study should have been repeated at the original doses to confirm by repetition the original results.

The anti-convulsant study showed that doses of 10 and 30 mg/kg ranolazine increased the lethality of seizures(80% and 90% respectively compared to 40% for the control group) At 80 and 90 mg/kg the tonic extensor seizures were incomplete. The animals were also observed to be sedated at these doses. At 100 mg/kg the seizures were absent (0/10).
Low doses of ranolazine appeared to increase the lethality of seizures while doses that caused signs of sedation decreased the incidence of induced seizures.
There was no apparent effect upon oxotremorine-induced tremors.

Gastrointestinal transit time data was presented in a somewhat undetailed report that makes it difficult to evaluate the data. No positive controls were presented and no results except for the sponsor's statement that the geometric center for the controls was 6.38±0.34 while the GC for the drug-treated group was 6.05±0.66. This result is difficult to interpret with no comparator compounds.

Pulmonary effects were examined over 15-15000 μg/kg. No positive control data was presented, no error bars were present on the graphs and no tabular data was presented, only graphical. The system showed a great deal of variability which confounds interpretation. The sponsor concluded that there were no significant differences from control. Given the cardiovascular effects that have been demonstrated to occur within the dose range used, secondary pulmonary effects might reasonably be expected.

No renal pharmacology data was presented.

No abuse potential data was presented.

Safety pharmacology conclusions: As tested and reported in the studies provided, ranolazine appears to have:

1) **significant cardiovascular liability**
2) **several central nervous system effects that include: a central sedative effect, the ability to induce neurologic deficits in the absence of sedation, increasing the lethality of induced seizures at low doses and some effect on the HPA axis that is manifested as an exaggerated response to stress.**

The negative inotropic effects seen in the cardiovascular safety study are comparable to calcium channel blocking effects. This raises a concern for the clinical use of this drug in patients with congestive heart failure where one might expect to see a marked decrease in contractility and left ventricular function (Goodman and Gilman's 10th edition).

We know nothing about the contribution of the many metabolites to these results. There is no margin of safety between the plasma levels at which these effects occurred in the animals and the therapeutic levels achieved in humans.

III. PHARMACOKINETICS/TOXICOKINETICS:

*The data for the human plasma levels was obtained from Peter Hinderling, M.D., the biopharmaceutics reviewer for this NDA.

PK parameters:

Absorption:

Absorption and excretion studies in dog following oral and intravenous administration of ^{14}C-RS 43285 at 5 mg/kg AT3407/SS/038/85 Sept 1984.

Male Beagles were given either an oral or an intravenous dose of ^{14}C-RS-43285-193 at 5 mg/kg. Blood, urine and feces were collected at unspecified times and radioactivity determined by tlc, HPLC and liquid scintillation counting.

Results: Peak plasma levels of radioactivity were reached within 30 minutes in 3 dogs and by 90 minutes in the 4th dog. Mean urinary excretion was 41% and 37% following oral and intravenous dosing respectively. Mean fecal excretion was 56% and 59% following oral and intravenous administration respectively. Mean total excretion of dosed radioactivity in urine and feces of 97% and 96% were obtained following oral and intravenous administration respectively. The ratio of oral to intravenous AUC values show mean bioavailability of >80% for the radioactivity and 17% for RS43285.

		Radioactivity			RS 43285		
Dog No.	Route of Administration	C_{max} (ng equiv.ml^{-1})	t_{max} (h)	AUC (0-infinity) (ng equiv.h.ml^{-1})	C_{max} (ng.ml^{-1})	t_{max} (h)	AUC (0-12 h) (ng.h.ml^{-1})
M4CA1	oral	3551	0.5	11451	146	0.5	99
M4CA2	oral	1854	1.5	9605	163	0.5	326
M4CA4	oral	3060	0.5	14831	372	0.25	520
M4CA5	oral	2788	0.5	9094	380	0.25	439
M4CA1	intravenous	-	-	12690	-	-	1373
M4CA2	intravenous	-	-	10824	-	-	2146
M4CA4	intravenous	-	-	11194	-	-	1995
M4CA5	intravenous	-	-	8066	-	-	1512

Summary: Consistent with other studies, ranolazine appears to be rapidly absorbed and excreted. The difference in bioavailability of the radioactivity and the drug is not clear, but contribution of metabolites may be involved.

^{14}C-RS 43285: Oral and intravenous absorption and excretion studies in the rat at 5 and 250 mg/kg AT3413/SS/020/85 July 1985

Male and female Sprague-Dawley rats were given 5 mg/kg oral and intravenous doses of radio-labeled ranolazine. Male rats also received 250 mg/kg oral doses of radio-labeled ranolazine. Blood, urine and feces were collected. Quantitation of radioactivity was by HPLC with a radioactivity monitor and an integration program.

Results: Atypical plasma profiles from 1 male and 1 female were omitted. Partial subcutaneous administration was suspected. After 5 mg/kg, Cmax was achieved within 30-60 minutes of oral

dosing. Mean urinary excretion accounted for 56 and 57% of dosed radioactivity after oral and intravenous administration respectively in male rats and 47 and 54% respectively in female rats. Rapid absorption was reported after the 250 mg/kg dose with a plateau in plasma levels from 1 to 6 hours. Urinary excretion accounted for 54% of dosed radioactivity. The sponsor's PK data is shown below.

Summary of PK data

Sex + N	Target dose Mg/kg	Route of Administra	Mean $AUC_{0-\infty}$ equiv.h.ml^{-1}	Mean Cmax (g equiv.g^{-1})	Median t_{max} (Hour)
M , 4	5	Oral	7.3	1630	0.50
F,4	5	Oral	5.6	1209	0.97
M,4	250	Oral	450.9	39468	3.85
M,3	5	IV	17.2		
F,3	5	IV	12.6		

SUMMARY OF PLASMA LEVELS (mcg/ml) IN MALE AND FEMALE RATS 1 HOUR POST-DOSE AFTER 1, 3 AND 6 MONTHS ADMINISTRATION OF RANOLAZINE AT 5, 50 AND 200 mg/kg

	Dose (mg/kg/day)		
Time Point	5	50	200
Males			
1 Month	0.803 ± 0.208 (0.559-1.34)	6.48 ± 2.24 (3.39-9.76)	18.4 ± 4.62 (12.1-25.9)
3 Months	0.724 ± 0.176 (0.352-0.896)	9.39 ± 3.12 (2.67-13.0)	22.2 ± 6.57* (15.4-35.8)
6 Months	1.02 ± 0.388 (0.721-1.92)	8.48 ± 2.32 (4.59-11.9)	36.7 ± 9.25* (23.6-48.4)
Females			
1 Month	1.39 ± 0.30 (0.945-1.89)	8.24 ± 2.42 (5.82-13.6)	24.4 ± 7.74 (12.7-33.5)
3 Months	0.998 ± 0.234 (0.690-1.33)	10.6 ± 3.41 (6.21-16.9)	32.4 ± 9.63 (20.2-46.6)
6 Months	2.91 ± 0.655* (2.16-4.29)	15.2 ± 4.59 (10.1-25.3)	43.7 ± 11.0 (33.8-66.3)

Values represent means ± S.D (Range)
n=10 except * where n=9

plasma exposure in rats orally dosed with ranolazine.

Evidence of absorption of ranolazine in the six month oral toxicity study in rats. AT5878, December 1991

Ranolazine in solution was given orally to male and female Sprague-Dawley rats at doses of 0, 5, 50 and 200 mg/kg/day for six months. Blood samples were collected at one month, three months and six months approximately 1 hour post-dose. Plasma levels of ranolazine were determined by HPLC.

Results: Plasma levels increased with increasing dose. Plasma levels increased in a relatively linear manner in males with the exception of the 3 month time point. At that time, the increase from 50 to 200 mg/kg was less than proportional. In the females, at all time points, the increase in plasma level from 50 to 200 mg/kg was less than proportional. Results are summarized in the sponsor's table below.

Since this is the comparison of plasma levels at one time point only, rather than a comparison of AUC values, all that can be said is that there was evidence of

Evidence of absorption of ranolazine in the six month oral toxicity study in dogs AT5879, December 1991

Ranolazine formulated in gelatin capsules was given to male and female Beagles at doses of 0, 5, 25 and 60 mg/kg/day once a day for 6 months. Blood samples were collected from all dogs days 43-46, days 106-109 and days 182-185. The collection times were before dosing, 30 minutes, 1, 2, 4 and 8 hours after dosing. Determination of plasma drug concentration was by HPLC methodology.

Results:

In both sexes, the increase in AUC with increasing dose was greater than proportional.

Reveiwer's Summary: Female PK Parameters

	Ranolazine Cmax (ng/ml)					
	5 mg/kg/day ***		25 mg/kg/day ***		60 mg/kg/day ***	
1 month	484±254		3580±1561		8858±1909	
3 months	710±124		4348±1078		10855±2713	
6 months	705±471		3958±895		9918±5096	
	AUC (ng.hr/ml)					
1 month	866±625	0.026x	7104±2081	0.211x	27430±8437	0.814x
3 months	1291±561	0.038x	8989±669	0.267x	27443±7945	0.814x
6 months	1399±702	0.042x	7361±1874	0.218x	27817±10337	0.825x

***multiple of human exposure

Reveiwer's Summary: Male PK Parameters

	Ranolazine Cmax (ng/ml)					
	5 mg/kg/day	***	25 mg/kg/day	***	60 mg/kg/day	***
1 month	712±246		3600±2423		8960±1060	
3 months	867±189		5170±1119		11895±2566	
6 months	697±217		3678±1185		9235±2040	
	AUC (ng.hr/ml)					
1 month	1343±361	0.040x	12318±2706	0.366x	28126±4265	0.835x
3 months	1720±202	0.051x	14364±4720	0.426x	37740±8458	1.120x
6 months	1655±388	0.049x	10319±3221	0.306x	29610±7995	0.879x

*** multiple of human exposure

Distribution:

^{14}C-RS-43285: Tissue distribution studies by whole body autoradiography in albino and pigmented rats RS-43285-193AT3356 April 1985

Albino Sprague-Dawley rats and Long Evans pigmented rats received a single oral dose of ^{14}C-RS-53285 dihydrochloride at 50 mg/kg. The albino rats were euthanized at 1,6 and 24 hours and the pigmented rats at 24 hours. Sagittal whole body sections were obtained at several levels throughout the carcass, exposed to radiograph film and contact prints developed to show distribution of radioactivity. It appears that there was one albino rat per time point and 1 pigmented rat at 24 hours.

Results: A visual discernment of distribution of radioactivity was made. At 1 hour, the highest apparent concentrations of radioactivity were in the GI tract, adrenals, kidney, lacrimal gland, liver, preputial gland and pituitary. The lowest levels were reported for the muscle, bone marrow, lung and testes. The sponsor states that no evidence of radioactivity was discerned in the brain or spinal cord. In the pigmented rat, levels of radioactivity in the eye were much greater than in the albino rat, possibly due to melanin binding. After 6 hours, levels of radioactivity in most tissues were apparently lower. By 24 hours post-dose, the highest levels of radioactivity were present in the contents of the stomach and large and small intestines. Levels of radioactivity were much greater in the eye of the pigmented rat than the albino rat. Because so few animals were studied, the report cannot be given too much weight.

^{14}C-RS-43285: Tissue distribution studies in the rat. RS-43285 AT3357, April 1985

Parallel studies with albino Sprague-Dawley rats and pigmented Long-Evans rats, n=4 per time point, were given a single oral dose of ^{14}C-RS-43285, 50 mg/kg. Rats were euthanized (AT3413) in groups of 4 at 1, 6, 24 and 72 hours after dosing. After euthanasia, thyroid, heart, lungs, testes, bone marrow, kidneys, liver, spleen, arterial wall, GI tract, xiphoid cartilage, fat, adrenals and brain were collected and weighed. Urine and fecal samples were collected daily from the group of rats euthanized at 72 hours.

Ocular studies: following a single oral dose of ^{14}C-RS-43285 at 5 mg/kg, Long Evans rats in groups of 2 were euthanized at 1, 2, 3, 7, 10, 14, 21 and 28 days post-dose. Control albino rats were euthanized at 1 and 2 days post-dose.

Quantitation of radioactivity was done by scintillation counting.

Results: Levels of radioactivity were reported to be highest at 1 hour and declined rapidly thereafter. In descending order, the principal tissue levels of radioactivity were found in the GI tract, liver, adrenals, kidney, thyroid, arterial wall, bone marrow, heart and brain. At 72 hours post-dose the radioactivity was principally associated with liver, kidney, GI tract and thyroid, although detectable levels were reported for all the original tissues.

Pigmented eyes at 1 hour contained 21.47 % dose x 10^{-3} vs 0.35% dose x 10^{-3} in the albino eyes. There was no detectable radioactivity in the albino eyes after day 2 while the pigmented eyes showed levels of 5.62 % dose x10^{-3}. The terminal half-life of elimination of radioactivity from the pigmented rat eye was ~ 23 days.

Mean urinary and fecal excretion accounted for 53% and 43% respectively of the dosed radioactivity.

Summary: Drug-derived radioactivity was found primarily in liver, adrenals, kidney thyroid and gastrointestinal tract. Drug-associated radioactivity was cleared rapidly from tissues with the exception of the pigmented eye. Skin was not discussed.

The tissue distribution of total radioactivity in the albino and pigmented rat following oral administration of [^{14}C]-ranolazine. December 1999-October 2001 CVT303.006R

Twenty nine male albino rats each received a single oral dose of [^{14}C]-ranolazine at a target dose level of 50 mg/kg. At each time point of 0.5, 1, 2, 4, 6, 8, 24 and 72 hours post-dose, 3 rats were euthanized and a number of tissues and/or blood collected. Urine, feces and cage wash were collected for the 5 remaining animals, housed in metabolism cages. The intervals for urine collection were: predose, 0-6, 6-24, 24-48, 48-72, 72-96, 96-120 hours. The intervals for fecal collection were: 0-24, 24-48, 48-72, 72-96 and 96-120 hours. The following organs were collected from the animals euthanized at 1, 6, 24, 72 and 120 hours post-dose: adrenals, bile duct, blood, bone mineral and marrow, brain, eyes, fat, harderian gland, heart, kidneys, large intestine, liver, lungs, muscle, pancreas, plasma, carcass, salivary gland, skin, small intestine, spleen, stomach, testes, thyroid, urinary bladder. Blood only was collected from rats in the 0.5, 2, 4 and 8 hour groups. Levels of total radioactivity were determined for each tissue and sample of excreta by liquid scintillation counting and combustion analysis.

Another 6 male pigmented rats each received a single oral dose of [^{14}C]-ranolazine at 50 mg/kg. One rat was euthanized at each of 6 timepoints: 24, 72, 168, 336, 504 and 672 hours post-dose. Levels of radioactivity were determined in one of the eyes and a blood sample by combustion analysis and liquid scintillation counting. Quantitative whole body autoradiography was also performed.

Results:
Albino rats: Mean total recovery of radioactivity over 120 hours was 98% of administered dose. Elimination was primarily through the feces with a mean of 53% (range 47 –62%). Elimination via the urine was on average 39%(range 30-43%). Cage washings accounted for 3-7% of radioactivity. 87% of the radioactivity was recovered within the first 24 hours after dosing.

The radioactivity was quickly distributed to every organ sampled. There were detectable levels of radioactivity in the adrenals, brain, eyes, heart and kidneys (and other organs) to 120 hours after dosing. The quantitative whole body autoradiography was not as sensitive. However, there were detectable levels of radioactivity in testes, adrenals and eyes.

Table 2 ***Mean Concentration of Total Radioactivity in Tissues Following Single Oral Administration of [^{14}C]-Ranolazine to Male Albino Rats. Target Dose Level: 50 mg.kg^{-1}***
Results expressed as µg equiv.g^{-1}

Sample	Timepoint									
	1 h Mean	1 h SD	6 h Mean	6 h SD	24 h Mean	24 h SD	72 h Mean	72 h SD	120 h Mean	120 h SD
Adrenals	46.70	5.10	6.75	2.59	1.52	0.22	0.61	0.02	*0.37	*0.12
Bladder	43.03	32.11	13.85	8.17	7.25	10.34	0.24	0.04	*0.14	*0.09
Bone Marrow	39.39	16.66	6.31	3.57	1.09	0.57	*0.09	*0.12	*0.11	*0.08
Bone Mineral	10.24	1.849	2.50	0.78	*0.42	*0.03	*0.09	*0.09	*0.13	*0.05
Brain	5.26	0.94	1.54	0.21	0.28	0.11	*0.05	*0.04	*0.06	*0.03
Eyes	9.80	1.90	2.10	0.25	0.22	0.06	*0.06	*0.01	*0.04	*0.03
Fat-Subcutaneous	12.36	0.83	1.50	0.28	0.69	0.45	0.25	0.03	0.20	0.05
Harderian Gland	111.64	36.68	16.96	6.40	2.19	0.60	0.39	0.05	*0.16	*0.07
Heart	22.49	2.91	2.68	0.27	*0.37	*0.09	0.16	0.01	0.12	0.03
Kidneys	60.80	8.36	11.98	0.73	2.58	0.73	0.91	0.03	0.57	0.17
Liver	138.62	24.60	22.76	7.75	4.36	1.30	1.51	0.20	0.83	0.22
Lungs	45.78	10.63	3.73	0.60	0.79	0.38	*0.17	*0.15	0.17	0.05
Muscle	14.72	1.80	2.25	0.31	0.36	0.14	0.13	0.01	0.13	0.01
Pancreas	36.05	15.17	4.01	0.34	0.57	0.18	0.25	0.03	0.17	0.04
Salivary Gland	44.41	1.57	7.36	1.71	0.72	0.28	0.20	0.03	0.17	0.02
Skin	17.62	1.22	4.24	0.44	0.64	0.07	0.40	0.08	0.27	0.01
Spleen	41.78	8.87	3.68	0.71	0.60	0.13	0.29	0.02	0.24	0.02
Testes	9.38	1.85	2.67	0.15	0.64	0.32	0.11	0.03	0.09	0.01
Thyroid	33.42	2.76	3.46	0.71	2.16	0.40	0.47	0.05	*0.63	*0.35
Bile duct	36.52	50.57	13.15	8.34	4.35	N.A.	*0.29	*0.25	*0.21	*0.20
Stomach	335.40	208.23	13.61	7.46	33.81	44.67	1.86	2.26	0.12	0.00
Stomach Contents	2181.50	785.83	14.88	6.33	78.52	100.50	4.76	4.23	*0.02	*0.02
Small Intestine	261.47	27.16	57.00	5.63	15.93	9.16	0.76	0.47	0.12	0.02
Small Intestine Contents	580.04	158.85	72.84	8.77	30.28	19.38	1.22	0.96	0.07	0.02
Large Intestine	35.49	9.49	341.52	67.94	36.61	11.34	1.90	1.07	0.14	0.03
Large Intestine Contents	20.01	3.13	391.47	53.49	50.02	22.23	2.24	1.22	0.09	0.03
Carcass	11.51	2.70	2.84	0.96	0.60	0.28	0.40	0.28	0.33	0.18
Whole Blood	14.31	2.31	2.92	0.38	0.55	0.17	0.18	0.01	0.12	0.02
Plasma	17.91	2.06	3.37	0.46	0.58	0.19	0.43	0.33	0.07	0.01

Values are mean and SD of data from 3 (1,6,24 and 72 h timepoints) or 5 (120 h timepoint) rats.
N.A.= Not available/analysed
*=Mean includes results calculated from data less than 30 d.p.m above background

Table 4 ***Concentration of Total Radioactivity in Tissues (QWBA) Following Single Oral Administration of [^{14}C]-Ranolazine to Pigmented Rats. Target Dose Level: 50 mg.kg^{-1}***

Results expressed as µg equiv.g^{-1}

Tissue	001M	002M	003M	004M	005M	006M
	24 h	72 h	168 h	336 h	504 h	672 h
Adrenal Gland	2.1	1.1	NM	NM	NM	NM
Bladder	NM	NM	NM	NM	NM	NP
Blood	NM	NM	NM	NM	NM	NM
Bone Marrow	1.6	NM	NM	NM	NM	NM
Brain	NM	NM	NM	NM	NM	NM
Brown Fat	1.9	NM	NM	NM	NM	NM
Epididymis	NM	NM	NM	NM	NM	NM
Eye	102.7	53.9	37.4	9.5	16.3	6.1
Harderian Gland	6.1	NM	NM	NM	NM	NM
Heart	NM	NM	NM	NM	NM	NM
Kidney	4.0	2.5	1.1	0.2	NM	NM
Lachrymal Gland	NM	NM	NM	NM	NM	NM
Large Intestine Wall	NM	NM	NM	NM	NM	NM
Liver	6.8	2.4	0.8	0.1	NM	NM
Lung	NM	NM	NM	NM	NM	NM
Lymph Node	NM	NM	NM	NM	NM	NM
Pancreas	1.3	NM	NM	NM	NM	NM
Pituitary Gland	NM	NM	NM	NM	NM	NM
Preputial Gland	2.5	1.1	NM	NM	NM	NM
Prostate	NM	NM	NM	NM	NM	NM
Rectum	NM	NM	NM	NM	NM	NM
Salivary Gland	1.6	NM	NM	NM	NM	NM
Seminal Vesicles	NM	NM	NM	NM	NM	NM
Skeletal Muscle	0.7	NM	NM	NM	NM	NM
Skin (Albino area)	0.8	0.7	NM	NM	NM	NM
Skin (Pigmented area)	15.0	7.5	NM	NM	NM	NM
Small Intestine Wall	NM	NM	NM	NM	NM	NM
Spinal Cord	NM	NM	NM	NM	NM	NM
Spleen	1.4	0.9	NM	NM	NM	NM
Stomach Wall	NM	NM	NM	NM	NM	NM
Testis	0.9	0.3	NM	NM	NM	NM
Thymus	NM	NM	NM	NM	NM	NM
Thyroid Gland	NM	NM	NM	NM	NM	NM
White Fat	0.2	NM	NM	NM	NM	NM
Limit of Reliable Measurement	0.3	0.3	0.3	0.3	0.2	0.3
Eye*	47.4	36.3	16.5	9.5	7.2	4.6
Blood*	0.5	0.2	0.1	0.1	0.1	0.1

* = Measured by combustion and LSC
NM = Not measurable
NP = Not present

Pigmented rats: The eye contained the highest concentrations of total radioactivity. The rate of elimination from both skin and eyes appeared to be slower than other tissues. The concentration in the eye at 28 days after dosing was ~6% of that measured at 24 hours post-dose (p.225, vol 42).

Concentrations in the eye were reported to diminish with a half life of ~ 8 days. Although it was a very small sampling of pigmented rats, the results are suggestive of melanin binding. This has not been confirmed with in vitro studies nor

described to any greater extent. Neither potential toxic effects upon the visual system nor potential for phototoxicity have been explored.

Metabolism:

Pharmacokinetics of ranolazine in male guinea pigs after a single intravenous dose of ranolazine at 6 or 30 mg/kgCVT303.008-R August-October, 2000. Reported: July 17, 2002

This is an amendment to the original report. Male guinea pigs, surgically cannulated, were given single intravenous doses of either 6, 30 or 60 mg ranolazine dissolved in saline. Blood was collected via the canulas at 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8 and 24 hours after dosing. Plasma concentrations of ranolazine and 3 metabolites found in humans (RS-88390 (CVT-2514); RS-88640(CVT-2512); RS-94287(CVT-2738)) were determined by LC/MS/MS. The range of quantification was listed as 50 – 10,000 ng/ml for ranolazine and 10 – 10,000 ng/ml for the 3 metabolites. The method was referenced to a paper regarding the use of human plasma. There is no indication that this assay was validated for guinea pig plasma.

Results: No adverse effects were reported for the 6 mg/kg dose. At 30 mg/kg, signs included hyperactivity, hyperventilation, tremors and prostration. These were reported to resolve by 1 hour. The 60 mg/kg dose was lethal. No further details were given regarding the HD effects. The pharmacokinetic parameters that were determined are shown in the sponsor's table below.

Summary of PK values

Dose(mg/kg)	6	30
Number of animals	2[a]	3[b]
$AUC_{(0-\infty)}$(ng.hr/ml)[c]	1089	6340±2010
CLp (ml/min/kg)	90.7	72.2±23.2
Vdβ(l/kg)	6.36	5.61±2.11
Elimination $t_{1/2}$	nd[d]	0.89±0.05[c]

[a] mean of 2 animals [b]Values are mean and SD. [C]- values were $AUC_{(0-t)}$ for the 6 mg/kg dose. [D]nd=not determined

[e]-half life determined on plasma concentrations between 1 and 4 hours.

The 3 specified metabolites were present in guinea pig plasma. These metabolites result from N-dealkylation at the piperazine ring, O-demethylation of the methoxyphenyl group and O-dearylation of the methoxyphenyl group. It is not specified in the report if the sponsor looked for other metabolites also.

Summary of metabolite data: AUC ng.hr/ml

metabolite	6 mg/kg	30 mg/kg
RS-88390	161	275
RS-94287	57.8	847
RS-88640	33.7	417

Given the low numbers of animals used for this study, limited conclusions may be drawn. It may be said that following single intravenous doses of ranolazine, the above 3 metabolites were found in guinea pig plasma.

Effect of RS43285 on hepatic drug metabolising activity in the rat and mouse. AT3397/SS/011/85, July 1985.

Several approaches were taken:

3. Male CD-1 mice received oral doses of phenobarbitone 250 mg/kg or ranolazine 250 mg/kg once a day for 5 days.
There were 10 mice per group (control, phenobarbitone and RS-43285). Day 6, the mice were given an intraperitoneal injection of pentobarbitone(70 mg/kg). Induction time was described as time from injection to the loss of righting reflex. Sleeping time was the time between loss and regaining the righting reflex.
4. Male Sprague Dawley rats (n=3 per group) were given oral doses of 250 mg/kg/day ranolazine or a saline vehicle once a day for 5 days. The rats were euthanized 24 hours after the last dose. Livers were collected and microsomal protein, microsomal CYP450 content and flavoprotein reductase content determined.
5. A single oral dose of saline, 25 mg/kg or 100 mg/kg of RS43285 was given to male rats one hour prior to an intraperitoneal dose of pentobarbitone (65 mg/kg). One hour later, the rats were given an intraperitoneal injection of pentobarbitone (65 mg/kg). Induction time and sleeping time were measured as above.
6. Control male rat livers were used for in vitro evaluation of potential inhibitory activity of RS43285 (0, 10, 50, 100 and 500 μM) on aminopyrine N-demethylase, biphenyl 4-hydroxylase and ethoxyresorufin O-deethylase.
7. NADPH-cytochrome c reductase activity was measured over a microsomal protein concentration range of 0.5-2 mg/ml.

Results: There was no significant difference between control and ranolazine for induction and pentobarbitone-induced sleeping time in mice. Liver weight and microsomal protein content were significantly increased in ranolazine-treated rats.

Effect of Chronic Oral Administration of RS 43285 to Male Rats on Some Indices of Hepatic Phase I Drug Metabolising Activity (DMAE 37))

Dosed Group	Final Body Weight (g)	Final Liver Weight (g)	Microsomal Protein Content[1]	Specific Microsomal Cytochrome P-450 Content[2] (uncomplexed)	Total Cytochrome P-450 Content[2] (complexed plus uncomplexed)	Microsomal NADPH-Cytochrome c Reductase Activity[3]
Control	221.7 ± 3.8	9.1 ± 0.7	11.7 ± 0.1	0.60 ± 0.02	ND	45.7 ± 3.8
RS 43285	228.3 ± 8.7	10.5 ± 0.6*	13.1 ± 0.2*	0.54 ± 0.02	0.55 ± 0.00	51.2 ± 6.7

RS 43285 was dosed orally at 250 $mg.kg^{-1}.day^{-1}$ for 5 consecutive days.

Results are given as the mean ± S.D. for three determinations. The statistical difference between the means was determined by the Student's t-test.

* Values statistically different from control ($P < 0.05$).

[1] mg protein.g $liver^{-1}$
[2] nmol.mg $protein^{-1}$
[3] nmol.mg $protein^{-1}.min^{-1}$
ND = not determined

There was significant inhibition of ethoxyresorufin O-deethylase activity and biphenyl 4-hydroxylase (BPH) at 100 and 500 µM. The inhibition of BPH was 35 and 48% mean inhibition at those 2 concentrations, respectively.

Ranolazine produced a slight increase in sleeping time acutely at 100 mg/kg. Chronic ranolazine administration caused a slight decrease in sleeping time.

Pentobarbitone induction and sleeping times

	ranolazine: acute administration		ranolazine:chronic administra	
	Induction time (minutes)	Sleeping time (minutes)	Induction time (minutes)	Sleeping time (minutes)
Control	2.6±0.5 (n=6)	135±18	2.7±0.6	56.2±14.5
Ranolazine 25 mg/kg	3.2±1.2 (n=5)	129±15	2.8±0.6	45.7±11.9
Ranolazine 100 mg/kg	2.2±0.3 (n=6)	144±18	6.0±2.8*	19.5±7.3#

*statistically different from control $p<0.05$; # $p<0.001$

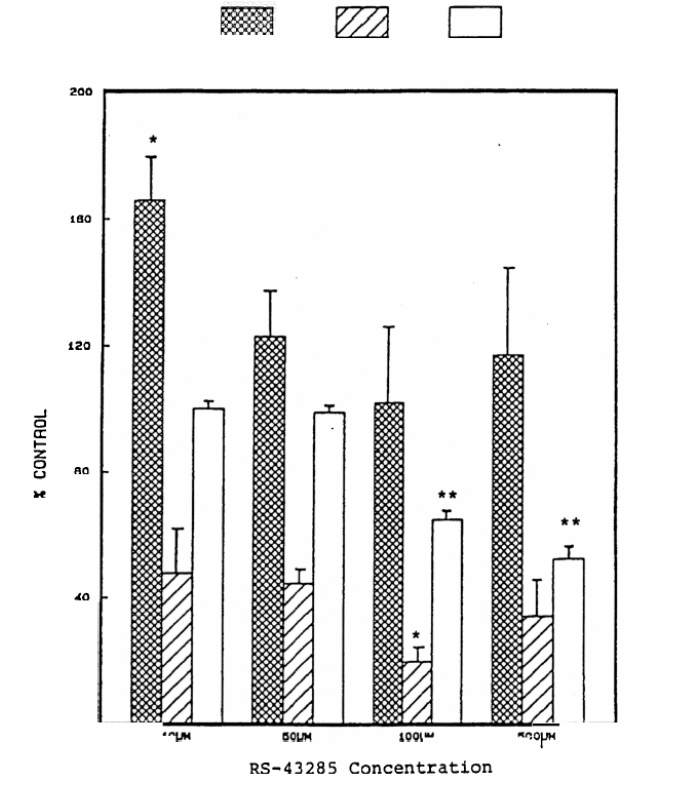


Figure 2 Effect of RS-43285 Added In Vitro on the Activity of Three Microsomal Drug Mono-oxygenases in Rat Liver.
APD- Aminopyrine N-Demethylase; EROD- Ethoxyresorufin O-Deethylase; BPH- Biphenyl 4-Hydroxylase
* $P < 0.05$; ** $P < 0.01$.

The hepatic rate of RS43285 metabolism: an in vitro assessment. AT3474. September 1985. The racemate RS 43285-193 (lot 125SS0584), the R enantiomer RS 43285-198 (lot number 5282-27) and the S enantiomer RS 43285-197 (lot number 5325-32) were compared against nicardipine hydrochloride. Male Sprague-Dawley rats and male CD-1 mice (both species from Charles River) and male Beagles (Balbeggie Kennels, Fife, UK) were used. After euthanasia, livers were collected from each animal and post mitochondrial supernatants prepared. Triplicate incubations for each condition were prepared. Samples were taken from each reaction mixture at time intervals up to 2 hours then processed for and analyzed by HPLC for parent compound levels. Unfortunately, the report does not specify how many animals per species were used nor the concentrations tested. One statement made in the results section suggests that only 10 µM and 100 µM concentrations were tested for one of the 3 compounds under consideration. We do not know about the usage of nicardipine.

Results The results are presented as 1)a table of rate constants for 2 concentrations of RS43285 with no indication as to whether this was the racemic mix or one of the enantiomers; 2) "An interspecies comparison of the relative in vitro rates of metabolism of nicardipine and RS43285 by liver post-mitochondrial supernatant" presents rate constants for 100 µM nicardipine and 100 µM RS43285 for the rat and dog; 3) a third table gives rate constants for 10 µM of the

enantiomers in rat liver(R: 1.20±0.27 h^{-1}; S: 1.08±0.18h^{-1}; racemate: 0.60 h^{-1}) 4) the 4th table gives reaction velocity (µg RS43285.g liver-1.min-1) for 0.103, 1.03 and 10.3 mM RS43285. The lack of data for multiple concentrations, lack of data for the enantiomers versus the racemic mix and the different concentrations reported for different species make it very difficult to extract useful information from this report. The study is inconclusive.

A further investigation of the potential inhibitory effect of RS 43285 on hepatic drug metabolising activity in the rat. Addendum to SRS Report No. SS/011/85, AT3619 June 1986

Male Sprague-Dawley rats (Charles River) were used as a source of hepatic microsomes. Microsomal ethoxyresorufin O-deethylase (EROD) activity was measured. Apparent Km and Vmax were determined for RS43285 concentrations of 0, 0.01, 0.05 and 0.10 mM using 7 substrate concentrations somewhere over the range 0.01-5 µM. Biphenyl 4-hydroxylase (BP4H) activity was also determined with apparent Km and Vmax determined for 4 concentrations of RS 43285 (0, 0.01, 0.05 and 0.10 mM) using 5 substrate concentrations over the range 0.01- 2 mM biphenyl. For unexplained reasons, control rat liver microsomes were used at 0.34 mg protein/incubation for the EROD assays and 2.0 mg protein. m^{-1}/incubation for the BP4H assays. The difference in designations of concentrations are the sponsor's.

Results: Vmax and Km increased slightly from 0 - 0.05 mM RS43285 for ethoxyresorufin O-deethylase. Vmax decreased slightly for biphenyl 4-hydroxylase with increasing concentration of RS43285. This is summarized in the reviewer's table below.

RS43285 conc (mM)	Ethoxyresorufin O-deethylase		Biphenyl 4-hydroxylase	
	Vm#	K_M µM	Vm*	K_m mM
0	128(91.7-213)	0.26(0.14-0.50)	2.63(1.33-8.33)	0.47(0.16-2.00)
0.01	167(122-270)	0.21(0.12-0.41)	3.03(1.33-33.33)	1.00(0.33-10.00)
0.05	196(130-385)	0.45(0.25-1.11)	1.26(0.90-2.85)	0.19(0.06-0.58)
0.10	169(115-333)	0.43(0.23-1.00)	0.95(0.53-2.22)	0.20(0.07-0.62)

pmol.min^{-1}.mg $protein^{-1}$ *nmol.min^{-1}. mg $protein^{-1}$ Values in parentheses are sponsor's 95% confidence intervals

CYP1A1 and CYP1A2 both metabolize 7-ethoxyresorufin. The specificity of biphenyl 4-hydroxylase is not mentioned in the report.

Summary: From the given data, it appears that under the conditions of the assay there was minimal effect of RS43285 on activity of either ethoxyresorufin O-deethylase or biphenyl 4-hydroxylase.

An in vitro assessment of the sites of metabolism of RS 43285 in the rat. AT3867 April 1987
Male Sprague-Dawley rats(Charles River) were euthanised and the liver, lungs, kidneys and an unspecified portion of the small intestine were collected. Post-mitochondrial supernatants were prepared from each tissue. Samples were incubated for intervals up to 1 hour. Concentrations and possible variations on chemicals used were not specified. After the metabolic reaction, protein was precipitated and the supernatant analyzed by HPLC for parent compound. The sponsor presents a single table of data, with each value from a single rat, showing rate constants

for RS43285 metabolism. Data is shown only for the liver and 1 value for the kidney with the note that no metabolism was detected in any other sample. As presented, the reviewer cannot come to an independent interpretation of the data.

The isolation and characterization of the metabolites of ranolazine in the rat. AT5956/ SS/020/89 February, 1992.
Male Sprague-Dawley (Crl:CD(SD)BR) rats (Charles River, UK) were orally gavaged with 250 mg/kg of ranolazine and placed in metabolism cages for separate collection of urine and feces. Urine collected was pooled to give a 0-24 hour sample.

Other rats were anesthetized and the bile ducts cannulated. After a recovery period the rats were orally dosed with 200 mg/kg. Bile was collected for 24 hours post-dose.

Semi-preparative and analytical HPLC were performed.

In vitro metabolism studies were also performed using ^{14}C-ranolazine in a buffered incubation system containing an S9 fraction prepared from rat livers. The samples were analyzed for metabolites by HPLC. Fractions from the semi-preparative hplc of the urine and bile fractions were hydrolyzed with glusulase. Mass spectrometry was performed on all prepared samples.

Results: There was no tabular summary of distribution of the metabolites only a textual presentation of the results. It is not clear from the report how many animals were used to generate the data. One comment in the report states that the bile data came from 1 rat. It was also stated that overall recovery of radioactivity was >70%. The sponsor proposed that the 31% of the total radioactivity remaining after extraction of the urine sample was conjugated material. The report also states that the hplc systems used failed to fully separate the monohydroxylated metabolites RS-88597, RS-88772/88835 and RS-89961 as well as the positional isomers RS-89664, RS-88597, RS-89356 and RS-89649 (3,4,5 and 6–monohydroxylated respectively in the methoxyphenyl ring). HPLC evidence suggested that only RS88597 was formed in vivo.
The most abundant metabolite in rat urine was RS-89289, accounting for ~9% of the dose. RS-94287 and a glucuronide conjugate of RS-88390were next most predominant at 6% and 5% of the dose respectively. All other components were reported to be present at ≤ 4%.

Metabolites in the bile of the one rat were reported to be exclusively conjugated species including the glucuronide conjugate of RS-88390 (16%) and sulfate conjugates of RS-88597 and RS-88640. Quantitation was confounded by co-chromatography.

The in vitro sample (n=1, p.164) showed ranolazine and 5 major metabolites as well as a number of minor metabolites. Potential stereoselectivity in the metabolism of ranolazine was not investigated in the present study. Metabolites were tentatively identified as RS-88250 and/or RS88755, RS88640, RS94287 and RS88597.

There is insufficient data presented to allow for an independent review of the material. A reasonable interpretation of the data is that certain metabolites were identified in the urine and bile of an unspecified number of male rats.

The identification of the metabolites of ranolazine in the rat. AT7014,May –Sept, 1994. Reported: January, 1995

THE ABUNDANCE OF RANOLAZINE METABOLITES IN 0-24 h MALE RAT URINE

Metabolite	Abundance *
RS-89289	9%
RS-89983	3%
RS-94287	6%
RS-89961	2%
ranolazine	4%
RS-88250/88755 - conjugate	3%
RS-89983 - sulphate	3%
RS-91347 - conjugate	2%
RS-88390 - glucuronide	5%

* Expressed as a percentage of the dose

On average 43% of dosed material was excreted within 24 h of dose.

Al other metabolites present at 1% or less of dose

Thirty male and 30 female rats (Crl:CD(SD)BR, Charles River, UK) were given oral doses of 150 mg/kg. A group of 9 male and 9 female rats received a single dose of non-radiolabelled ranolazine Day 1 and another group (21 male, 21 female) received a single daily dose of non-radiolabelled ranolazine for 7 days with a further single dose containing ^{14}C-RS-43285 (15-20 µCi/animal) on Day 8. Blood samples were collected day 1 at 0.5, 2.5 and 5 hours after dosing. Day 8, blood samples were collected at 0.5, 1.0, 2.5, 5, 10 and 24 hours post-dose. Urine and fecal samples were collected from 3 male and 3 female rats placed in metabolism cages. The excreta samples were removed at 6, 24 and 48 hours after administration of the radiolabelled dose. Pooled samples of urine, feces and plasma were prepared for analysis by liquid scintillation counting, LC, NMR and MS.

Results

Plasma: Nine compound-related components were found in male rat plasma. Of these, 3 were each present at >10%.The significant metabolites were RS-91437 +RS-89983 (28% total), RS-88390 glucuronide (13%) and ranolazine (40%).

Six radiochemical components were found in female rat plasma of which 2 were considered significant. These were the glucuronide conjugate of RS-88390 (12%) and ranolazine (66%).

Urine: Eighteen compound –related components were reported for male urine samples. Three peaks were present at >10%: possibly RS-89289(20%), RS-91437(14%) and glucuronide conjugate of RS-88390(19%). Ranolazine was present at 5% of the urinary radioactivity.

Female urine contained 17 compound-related species. The greatest peaks were RS-91437(28%), RS-88390 glucuronide (21%) and ranolazine (23%).

Feces: Male feces contained 13 components with 3 significant: RS-88755(11%), RS-88597 (12%) and RS-88390 (29%). Ranolazine was present at 9%.

Female feces contained 25 peaks with 3 significant: RS-88250(17%), RS-88390 glucuronide (14%) and RS-88390 (20%). Ranolazine was present at 3% of total radioactivity in the fecal sample.

The study indicates sex-related differences in metabolism. The sponsor did not tabulate the relative abundance of the various metabolites in the different matrices but discussed selected metabolites in the text of the report. In the female rats, 5 hours post-dose, ranolazine accounted for ~2/3 of total circulating radioactivity compared to ~ 40% for male rats. Unchanged ranolazine was present in 0-24 hour female urine as ~23% of radioactivity compared to 5% in male urine. In male plasma, the major metabolite was RS-91347, with RS-89983 and the glucuronide of RS-88390 of approximately equal significance (to each other or to RS-91347 is not clear). In female rats, RS-91437 was the principal metabolite followed by RS-88390 glucuronide and RS-88597. RS-89289 was not identified in the female rat plasma.

The metabolism in both sexes appeared to be due predominantly to hydrolysis (RS-91437), O-demethylation (RS-88390) followed by glucuronidation and then N-dealkylation (RS-89983) which is further metabolised to RS-89289.

Effect of RS43285 on hepatic microsomal levels of cytochrome P-450 and protein in dogs following oral administration for six months. AT4031 March, 1988.

RS 43285 (lot 171SS0986), formulated with lactose and magnesium stearate in gelatin capsules was orally dosed to male and female Beagles (Alpha Sirius, Colwall, UK) at doses of 0, 5, 25 and 60 mg/kg/day for 6 months. After euthanasia, liver samples were collected from the control and 25 mg/kg animals. Microsomes were prepared and the microsomal protein content and CYP450 concentrations determined.
Results: There were no apparent differences in any of the parameters measured in either sex. The study would be more convincing if all dose groups had been examined or if at least the HD group had been examined also.

Effect of RS43285 on hepatic microsomal levels of cytochrome P-450 and protein in rats following oral administration for six months. AT4032, April 1988.

RS 43285-193(lot 153SS7085) was given orally to male and female Sprague-Dawley rats (Charles River, UK) at doses of 0, 5, 50 and 200 mg/kg, once a day for six months. After euthanasia, liver samples were collected from the control group and MD group. Microsomes were prepared and microsomal protein and cytochrome P-450 content determined.

Results: There was a 25% increase in microsomal CYP450 content in the males and a 26% decrease in the same parameter in females. The report would be more convincing if all dose groups had been sampled or at least the HD group had been included in the analysis.

The isolation and characterisation of the metabolites of ranolazine in mouse urine. AT6580, August 1989, Reported: February 1994

Six male mice in the three month oral dose ranging study were housed in metabolism cages and given a single oral dose of ^{14}C-ranolazine at 35 mg/kg. The urine was pooled by percentage volume (110 ml, 0-24 hours). Analysis of samples was by hplc and MS. Relative amounts of the metabolites were determined by liquid scintillation counting. For calculation purposes, the sponsor assumed that the total of the fractions isolated from mouse urine was equivalent to the mean percentage of the dose excreted in that sample:
[(radioactivity in isolated fraction)/(sum of radioactivity in all isolated fractions) x percentage of dose excreted in sample] = relative abundance

Standards used for metabolite identification were RS-88390, RS-88640, RS-89537, RS-89681, RS-89289, RS-89983, RS-89961, RS-88772, RS-88835, RS-88755, RS-88597, RS-89664, RS-88250, RS-91437, RS-89356, RS-89649 and RS-94287.

THE ABUNDANCE OF MAJOR RANOLAZINE METABOLITES ISOLATED FROM 0-24 h MOUSE URINE

Metabolite	Abundance in Urine % of dose	Fraction
RS-89983 Glucuronide	9%	Fr 2
RS-89289	25%	Fr 3*
Unidentified	4%	Fr 4
RS-94287	18%	Fr 5
RS-88390 Glucuronide	3%	Fr 6

Mean levels of 61.1% of the dose were excreted in urine in 0-24 h. Abundances are expressed as a percentage of the total radiochemical dose. All other components, including ranolazine, were present at less than 1% of the dose.

* multi-component fraction.

Results: An average of 61% of the radiochemical dose was eliminated in the urine in 24 hours, somewhat lower than the rat which averages ~90% in the first 24 hours. The major component identified in the urine was RS-89289. The sponsor's results are shown here.
Ranolazine was reportedly excreted as a minor component (<1% of dose, p 276).

It was proposed that the in vivo metabolism of ranolazine in the mouse was by one of two oxidative pathways: N-dealkylation or O-demethylation, sometimes followed by glucuronic acid conjugation. Hydroxylation did not appear to be a significant means of elimination of ranolazine in the mouse.

The metabolite profiles of ranolazine in rat, mouse, hamster and dog. AT6360, Conducted Feb.1987-Feb-1988. Reported: May 1993.

The report compares the metabolite profiles of ranolazine in various animal species as determined by hplc with radiochemical detection. Radioactive components were tentatively identified solely on the basis of chromatographic retention relative to ranolazine and using unlabelled standards of known molecular structure. The samples used for analysis were generated in studies reported elsewhere. The identification of those studies was:

DMAE 510/513 male and female rat plasma after single oral administration of ^{14}C-ranolazine.
Dose used 100mg/kg, n= 6 per sex

DMAE 517- male rat plasma after single oral administration of ^{14}C-ranolazine
Dose used 25 mg/kg, n=8

DMAE 467, 468, 472 male rat bile samples after single oral administration of ^{14}C-ranolazine
Dose used 5 mg/kg, n=4

The characterization of the dog metabolites was referenced to a report in preparation. The references for the rat studies are different than the designations that have been used in this NDA to identify the reports.

The standards used for identification of the metabolites were RS-89289, RS-89983, RS-88597, RS-88640, RS-94287, RS-88755, RS-88772, RS-88835, RS89664 and RS-88390.

Results: The sponsor notes that metabolite fractions do not necessarily represent discrete metabolite species but may represent heterogeneous mixtures of co-eluting components. The data is presented as representative chromatograms for the different species and matrices.

Reviewer's summary of textual description of results

Metabolites	20 minutes post-IV	6 hours post IV	Males		Females	
			1hr, po	6 hr, po	1hr,po	6hr,po
Ranolazine	61% total radioactivity		44%		58%	"similar"
RS89289	8		9%			
RS-89983	11	"almost solely"	27%	73%		
RS-88640	10		11%		36%??	"similar"
RS-88597						
RS-94287						
RS-88755						
RS-88772						
RS-88835						
RS-89664						
RS-88390						
RS-43285						

The sponsor states that there was no dependence on route of administration or dose in the observed metabolite profiles. There was insufficient data presented in the report to allow the reviewer to evaluate the statement. Ranolazine predominated at 1 hour post-dose as greatest percentage of total radioactivity (inconsistent with other reports that show a rapid disappearance of ranolazine). The major metabolites in males were the N-dealkylation products RS-89289, RS-88640 and RS-88640 (O-dealkylation). In female rats the sole entity other than ranolazine that was reported was RS-88640. The biliary results suggest " conjugated metabolite species."

Comparison of the in vitro metabolism of ranolazine in mouse, rat, dog and human liver microsomes. CVT303.005-MET, Jan-Feb 2002. Reported: July, 2002.

The sponsor states that both liver S9 and microsomes were used for the comparison of metabolism but only the microsomal data was reported. The rationale was that the data from the S9 and the microsomes was 'essentially similar."

Liver microsomes from male CD-1 mice, Sprague-Dawley rats, Beagles and humans were purchased from a commercial supplier. The protein content, total P450 concentrations and the activities of the major CYP450 isozymes were characterized by the vendor and not subsequently confirmed before use. Microsomal protein at presumably a final concentration of 0.20mg/l was used with ranolazine at a final concentration of 20 µM. A two minute preincubation was followed by the addition of NADPH to initiate the reaction. Incubation times were 5, 10,20, 30 and 60 minutes. LC/MS/MS analysis was used to assess disappearance of ranolazine and formation of metabolites. Quantification was by use of an internal standardization method. The sponsor defined "rate of formation" of metabolites as the maximal rate of appearance of individual metabolites between two subsequent time points.

Table 1 Comparison of Metabolites Formed in Liver Microsomes Prepared from CD-1 Mice, Sprague Dawley Rats, Beagle Dogs and Humans

Route/Metabolites	Maximal Rates of Metabolism of Ranolazine and Formation of Metabolites (pmole/min/mg microsomal protein) [a]			
	Mouse	Rat	Dog	Human
Total metabolism				
Ranolazine	894	753	491	589
N-dealkylation at the N4 piperazine nitrogen				
RS-94287	**145**[b]	**102**	**60.6**	**160**
CVT-2534	**65.8**	**78.6**	31.9	**99.5**
CVT-4786	10.5	4.43	7.46	**33.7**
N-dealkylation at the N1 piperazine nitrogen				
RS-88681	**441**	**150**	**81.7**	17.3
RS-89983	59.3	29.7	11.7	5.4
RS-89289	5.14	3.41	3.24	6.91
Hydroxylation at the dimethylphenyl ring				
RS-89961	**88.5**	30.0	21.5	**17.1**
RS-88772	12.6	3.71	7.58	8.67
RS-88835	48.6	23.2	**85.4**	7.00
Hydroxylation at the methoxyphenyl ring				
RS-88597	17.6	25.3	**128**	18.6
RS-89664	2.10	1.47	ND[c]	1.58
RS-89356	10.9	16.2	53.7	9.57
O-Demethylation				
RS-88390	19.1	**39.7**	50.6	**110**
O-Dearylation				
RS-88640	1.31	2.43	2.70	5.05
Amide bond cleavage				
RS-91347	2.04	2.14	1.47	1.09
Downstream metabolites				
Desmethyl RS-88681	6.35	28.4	0.794	1.17
RS-88755	2.00	13.2	0.447	0.886
RS-101647	2.04	1.53	0.307	0.882

[a] Values represent the overall metabolism (disappearance of ranolazine in incubate) and formation (appearance) of various metabolites.

[b] Values in bold represent the top five metabolites for each species.

[c] ND denotes not detected

Results: Except for RS-89664, not found in the dog microsomes, the 18 metabolites for which the sponsor analyzed were present in all 4 species. There were quantitative differences. These 18 Phase I metabolites are products of 11 metabolic routes. These 18 metabolites accounted for 86%, 90%, 92% and 79% of the total radioactivity in the mouse, rat, dog and human liver microsomes respectively.

For most of the primary metabolites, maximal rates of formation occurred between 0-5 minutes. Some metabolites exhibited higher rates of formation at later time points. One may ask whether this was real or an artifact of in vitro methods (e.g. such as the degree and uniformity of mixing).

Summary: Overall, there was a qualitative similarity between the in vitro results obtained between the 4 species examined in this study. A weakness of the study is that: 1) the sponsor did not confirm the total protein and microsomal information of the purchased materials. 2) the results presented contained no error bars on the graphs. The tabular results did not contain a ±SD. Were replicate samples processed or were results based upon N=1 per time point per species?

The isolation and characterisation of the metabolites of ranolazine in the dog. AT6377,Conducted January 1985-November 1992. Reported: May 1993.
The metabolism of ranolazine in the dog was investigated in vitro using S9 liver preparations and in vivo using urine and bile samples from dogs dosed orally with ranolazine 60 mg/kg. Metabolites were isolated by solid and liquid phase extraction followed by semi-preparative hplc. Mass spec methods were used on the radiolabeled fractions. Liquid scintillation counting was used for detection of radioactivity. Standards used for identification were RS-89289, RS-89983, RS-94287, RS-88640, RS-88250, RS-88755, RS-88835, RS-88772, RS-89961, RS-88681, RS-89537, RS-88597, RS-89664, RS-89649, RS-89356, RS-91347 and RS-88390.

Results: The sponsor's results are summarized in the table below:
Reviewer's condensation of sponsor's table summarizing abundance of major metabolites in dog urine/bile (pp. 178-179)

metabolite	Abundance in urine	Abundance in bile
RS-94287	3%	
RS-88640	6%	
RS-88597	1%	
RS-88835/88772 glucuronide	2%	
RS-88597 glucuronide*,**		
RS-88597 glucuronide*	1%	
RS-88390 conjugate	2%	
RS-88597 glucuronide	3%	
RS-88835/88772 conjugate	4%	
RS-88597 conjugate		
Ranolazine glucuronide	3%	
RS-88835/88772 sulphate		3%

* RS-88597 has the potential to form two glucuronide conjugates differing only in the position of conjugation. **The sponsor speculated that this peak may have been due to cross contamination from another peak.

The in vitro liver assay was textually described as producing 9 fractions, 4 of which were characterised against standards. The named metabolites, RS-94287, RS-88597, RS-88835/RS88772 and RS-88390 were reported to have been identified in rat in vitro studies.

Previous studies have indicated that approximately 30-32% of administered ranolazine is excreted in the urine and the remainder is excreted via the feces.

The identification of the metabolites of ranolazine in Baboon plasma AT6812,Conducted Oct.1993-Dec 1993. Reported: November 1994.

This report does not specify the number, age, sex or origin of the baboons used nor the rationale for the use of baboons. The in life methods were described as "sample origin". The present report states that "Full details of animals, dose preparation and administration and of samples collected are contained in the report pertaining to that study.[1]" The footnote was found to be listed as "Alps et al., (1991) SRS report no. SS/082/91. Reduction of myocardial enzyme release by RS-43285 (ranolazine) in a subhuman primate model of ischemia with reperfusion: post-reperfusion treatment with RS-43285-193 (racemate) and its enantiomers RS-43285-197 (S-isomer) and RS-43285-198 (R-isomer). The introduction states that the baboons had either undergone a 12 hour continuous iv infusion with either RS-43285-197 or RS-43285-198 at a target dose level of 50 μg/kg/hr following a loading dose of 500 μg/kg. Blood samples were "removed" 12 hours post-infusion and one sample analysed per animal as part of the metabolite characterisation.

Analysis of the one sample collected per animal was analysed by HPLC, MS and LC/MS. Control plasma samples were spiked with known amounts of standards for semi-quantitation of the metabolites found.

The protocol also states that liver microsomal and in vitro hepatocyte metabolism experiments will also be included in this study, possibly using ^{14}C-ranolazine. The protocol also states that blood samples will be taken at sequential times or at a single timepoint. Urine, feces and bile were also supposed to be collected. There appears to be some inconsistency between the written protocol and the data reported.

Results
Based upon the presentation of results, it appears that the baboons received either the racemic mix or one of the enantiomers. The baboon receiving the R-enantiomer showed at least 22 possibly drug-related components in the LC-MS analysis. Shoulders on a variety of peaks indicated the potential for other metabolites. Ten metabolites were positively identified. The analyses of plasma extracts from 3 baboons dosed with the S-isomer showed a similar wide range of metabolites. There were no significant qualitative differences apparent although there was some variation in absolute and relative peak intensities (p. 20).

The major plasma metabolite was RS-94287 (up to 29% of parent compound). Four other compounds at concentrations >10% of parent compound concentration were RS-88390 (16%), RS-89983(up to 13%), RS-88640(up to 12%) and RS-89961 (up to 11%).

The interanimal inconsistency of finding RS-89289 and RS-91347 was addressed by the sponsor as possibly due to the analytical method being suboptimal for these particular compounds. Given the uncertainty of the methodology, one may summarize this study by saying that single timepoint plasma samples from baboons given intravenous ranolazine show evidence of extensive metabolism. Both Phase I and Phase II metabolism are suggested. One may ask

whether a single sample taken 12 hours after the end of an intravenous infusion was the optimum methodology for examining the metabolic profile.

Excretion:

The disposition of ranolazine following oral administration of 50 mg.kg^{-1} [^{14}C]-ranolazine in the mouse CVT303.009-R May 17, 2002

A single oral dose of 50 mg/kg [^{14}C]-ranolazine (specific activity 925MBq/mmol) was given to 6 male CD-1 mice housed singly in metabolism cages. Urine was collected at 0-6, 6-24, 24-48, 48-72, 72-96 and 96-120 hours post-dose. Feces was collected 0-24, 24-48, 48-72, 72-96 and 96-120 hours post-dose. Twenty-four male CD-1 mice received single oral doses (as described above) for plasma level determination of drug. Terminal blood samples were collected at 0.5, 1, 2, 4, 6, 8, 24 and 48 hours post-dose. Levels of total radioactivity were determined for the plasma. RBCs were discarded. Liquid scintillation counting was used to quantify the radioactivity.

Results: Following oral administration the total radioactivity showed a peak mean plasma level at the first sampling time of 0.5 hours. The concentration of total radioactivity decreased to ~60% of peak levels by 1 hour. Total plasma radioactivity continued to fall as summarized in the reviewer's table below.

Reviewer's summary of total plasma radioactivity for mice given a single oral dose of [^{14}C]-ranolazine

Mean plasma total radioactivity (µg base equiv.ml^{-1})	Post-dose timepoint			
	0.5hours	1hour	24hours	48hours
	24.461	15.042	0.239	0.077

From vol 43, p.117

Approximately 30% of the total radioactivity was excreted in the urine in the first six hours. Urinary excretion accounted for 48.4±3.7% of the administered dose (drug or radioactivity not specified) and feces accounted for 45.8±2.7% over the collection period. Including gastrointestinal contents and carcass, 94% (range 91 - 97%) of the administered dose was recovered.

Summary: Peak plasma levels of radioactivity were achieved by the first sampling point of 0.5 hour. Excretion was approximately equally divided between urine (if cage wash was included) and feces and was essentially complete by 120 hours post-dose.

An investigation of the pharmacokinetics of ranolazine in the male rat following oral dosing for 7 days. AT6152, August, 1992.

Thirty male Sprague-Dawley rats were orally dosed at 50 mg/kg/day and blood samples from 2 rats per timepoint were collected day 7 at 10 min, 20 min, 30 min, 40 min, 1 hr, 1.5 hr, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours. Plasma drug levels were determined by HPLC. This is a rare study where a calibration range (10 – 1000ng) for ranolazine was mentioned.

The sponsor states that the purpose of the current study was to determine pk data after once daily dosing to steady state.

Sponsor's summary of PK parameters in male rats after 7 days of dosing.

Parameter	Male rat value
Cmax (ng/ml)	4130
Tmax(hour)	0.50
AUC_{0-24}(ng.h/ml)	18300
Clpo (ml/min/kg)	49.9

The sponsor does not present data to support that these are steady state values.
The sponsor cites AUC_{0-24} values for doses of 240, 320 and 400 mg given t.i.d to steady state (1992 SS/022/92 Safety and tolerability of multiple oral doses of ranolazine in healthy young male volunteers). This data was used to calculate the dose exaggeration in the animals and is summarized in the reviewer's table.

Reviewer's Summary of Relative Dose Exposure

Human dose (mg, tid)	Human AUC_{0-24}	Rat multiple of the human exposure
240	13200	1.4
320	19100	0.96
400	23100	0.79
1000	33700	0.54

The pharmacokinetics of ranolazine in male and female rats following single intravenous and single oral administration. AT6154,Conducted October 1987-March 1988. Reported: August 1992

Thirty male and 30 female rats were dosed intravenously at 25 mg/kg and 24 male and 24 female rats were dosed orally at 100 mg/kg. All doses were given as aqueous solutions (5%dextrose for iv, water for oral). Blood samples were taken from 3 animals/sex/timepoint at 5 min, 10 min, 20 min, 40 min, 1 hour, 2,3,4,6, and 8 hours after intravenous dosing and at 20 minutes, 40 minutes, 1 hour, 2,3,4,6 and 8 hours after oral dosing.

Results: Sex-related differences were apparent in the clearance values and subsequent exposure as shown by AUC. The females showed lower clearance after IV dosing but had lower AUC values. The sponsor's values are shown below.

PHARMACOKINETIC PARAMETERS OF RANOLAZINE FOLLOWING SINGLE INTRAVENOUS AND SINGLE ORAL ADMINISTRATION TO RAT

	INTRAVENOUS			ORAL	
Parameters	Male	Female	Parameters	Male	Female
C_0 (ng/ml)	16700	25200	C_{max} (ng/ml)	10800	19500
			t_{max} (h)	0.333	0.667
AUC_{0-inf} (ng.h/ml)	10700	30100	AUC_{0-inf} (ng.h/ml)	30400	76500
$t_{1/2}$ (h)	1.05	2.42	$t_{1/2}$ (h)	6.20	3.65
Systemic Cl (ml/min/kg)	38.9	13.8	Oral Cl (ml/min/kg)	54.8	21.8
Vd (L/kg)	3.54	2.89			
			ioavailability (%)	71.0	63.3

A study to investigate the pharmacokinetics of ranolazine in male and female rats following once daily oral dosing at 2,5, 50 and 150 mg/kg for 6 months. AT6811,Conducted Feb 1992. Reported: November 1994

Sixty-five male and 65 female Sprague-Dawley rats (Crl:CD(SD)BR, Charles River, UK) were dosed once a day by oral gavage for 6 months at the above-mentioned doses. Doses were based on daily weight for the first 10 weeks of the study and weekly weight thereafter. There were a number of rats dosed by oral gavage only on day 1, and at 1, 3 and 6 months, equivalent to 4 single oral doses. Blood samples were taken on day 1, and at 1,3 and 6 months at 0.5, 1.5, 3,6 and 24 hours after dosing. It is not clear from the report how many animals were sampled per time point. The summary indicates that blood was collected from 2 rats per time point. Plasma levels were determined by hplc methodology. The LOQ for the assay was 5 ng/ml.

Results: For both sexes, at all doses (both the multiple and single dose protocols), Cmax and AUC $_{0\text{-}24}$ increased over time. The only exceptions to this were the AUC values for the single dose males. The increases in AUC with increasing dose were greater than proportional in most cases. Results are summarized in the sponsor's table below. The apparent increase in exposure in the multiple dose rats may be due to accumulation, an age-related phenomenon or due to variability resulting from the small sample size.

Table 7
RANOLAZINE C_{max} AND AUC VALUES FOLLOWING MULTIPLE ORAL ADMINISTRATION AT 2, 5, 50 AND 150 mg/kg/day FOR SIX MONTHS AND SINGLE ORAL ADMINISTRATION AT 5 and 150 mg/kg ON DAYS 1, 32, 92 AND 183

Parameter	Dose	Females				Males			
		Day 1	Day 32	Day 92	Day 183	Day 1	Day 32	Day 92	Day 183
C_{max} (ng/ml) Multiple Dose	2 mg/kg/day	197	380	420	403	90.6	160	264	139
	5 mg/kg/day	548	974	933	1720	438	454	393	690
	50 mg/kg/day	4380	4960	5450	7020	2740	3920	4140	5160
	150 mg/kg/day	10000	13800	21200	15600	9840	13200	12200	19200
C_{max} (ng/ml) Single Dose	5 mg/kg	657	777	1230	1750	344	402	247	361
	150 mg/kg	15600	16700	11200	21200	14200	7980	8480	17000
$AUC_{0\text{-}24h}$ (ng.h/ml) Multiple Dose	2 mg/kg/day	464	840	943	1020	165	238	339	227
	5 mg/kg/day	1480	2220	2300	5000	660	936	1530	1260
	50 mg/kg/day	29000	36400	38900	50300	7210	12200	14100	22800
	150 mg/kg/day	99800	169000	126000	167000	59500	118000	63600	117000
$AUC_{0\text{-}t}$* (ng.h/ml) Single Dose	5 mg/kg	1360	1610	1830	3600	617	734	454	736
	150 mg/kg	136000	161000	114000	197000	77900	76700	54900	89100

* $AUC_{0\text{-}t}$ = $AUC_{0\text{-}6h}$ at 5 mg/kg and $AUC_{0\text{-}24h}$ at 150 mg/kg

Metabolic profiles of ranolazine following oral administration of a single 50 mg/kg dose of [^{14}C]ranolazine to male albino rats.CVT303.003-MET Amendment August, 2002

The stated reason for the amendment is to add metabolites that were either not detected or were below the quantification limit of the assay in the summary tables and footnotes.
Male Sprague-Dawley rats were given single oral doses of 50 mg/kg of [^{14}C]-ranolazine. Concentrations of total radioactivity in plasma and recoveries of the radioactive dose in urine and feces up to 5 days were determined. Details of the timepoints of collection of samples were not provided. Samples of plasma and urine were subjected to LC/MS/MS quantification for 18 metabolites. The details of the samples analyzed (time points) were not provided. These samples were also hydrolyzed with β-glucuronidase/sulfatase for estimation of conjugated metabolites.

Results: Peak concentrations of ranolazine and metabolites were reported to be reached within 1 hour. Peak concentrations for total radioactivity and ranolazine were reached 0.5 hour after dosing (p.257, vol 40). At Cmax, ranolazine accounted for 25% of total radioactivity in plasma, decreasing to 1% at 8 hours post-dose. Based on AUC_{0-24} ranolazine accounted for 13% of total radioactivity in plasma.

At least 40 metabolites were found in plasma and more than 80 were found in the urine. Of the plasma metabolites, 20 had AUC values >1% that of ranolazine. Approximately 9 had AUC values >10% that of ranolazine: RS-89983(113%), RS-91347 (46.3%), CVT-4786 (31.6%), RS-89289 (18.8%), RS-94287(15.3%), RS-89961(13.6%), RS-88390(16.3%) and ranolazine glucuronide conjugates(17.7%) and RS-88597 unspecified conjugate(10.6%) had AUC values > 10% that of ranolazine (p.258, vol 40).

Following oral administration an average of 39% and 53% of the radioactive dose was recovered in 5 days in urine and feces respectively. The 0-24 hour urine pool contained 96% of the radioactivity recovered in the urine. This pool was used for the metabolic profiling (a point mentioned in the results, not the methods section).

Ranolazine and the metabolites quantified accounted for 78.5% of the total radioactivity in urine. Of that , only 4% of the dose was recovered in urine as unchanged parent compound. The metabolites not quantified and unknown metabolites combined to account for the remaining 21.5% of total radioactivity in the urine.

CVT-4786 was listed as a major new metabolite identified in plasma and urine in this study. This was also mentioned in a similar dog metabolism study. This metabolite accounted for 9.4% of total radioactivity in urine.
Two N-oxides at the N-1 and N-4 piperazine positions were noted as identified for the first time in rats also in this study.

Concentrations of Total Radioactivity, Ranolazine and Metabolites in Pooled Plasma Following Oral Administration of a Single 50-mg/kg Dose of [^{14}C]-Ranolazine to Male Rats

(Concentrations of Ranolazine and Metabolites Determined by LC/MS/MS Method)

Time Point	Concentration in Plasma (µg Ranolazine Equivalent/mL for Total Radioactivity or µg/mL for Ranolazine and Metabolites)													
(hr)	Total ^{14}C	Ranolazine (% of Total ^{14}C)		RS-88390	RS-88640	RS-88681	RS-94287	RS-89983	CVT-2534	CVT-4786	Des-methyl RS-88681	RS-88755	RS-101647	RS-91347
0	BQL	BQL	-	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
0.5	20.8	5.23	(25.1)	0.172	0.043	0.269	0.730	1.42	0.437	1.64	0.474	0.072	0.070	1.88
1	17.9	3.01	(16.8)	0.107	0.040	0.208	0.631	1.69	0.317	1.38	0.364	0.069	0.071	1.48
2	11.9	2.21	(18.5)	0.097	0.034	0.084	0.328	1.41	0.127	0.505	0.158	0.047	0.032	1.13
4	6.04	1.29	(21.4)	0.063	0.020	0.027	0.167	0.853	0.045	0.275	0.049	0.042	0.012	0.666
6	3.37	0.112	(3.3)	0.010	BQL	BQL	0.030	0.729	0.010	0.084	0.012	0.010	BQL	0.064
8	2.33	0.027	(1.2)	BQL	BQL	BQL	BQL	0.511	BQL	0.025	BQL	BQL	BQL	BQL
24	0.582	BQL	(0.0)	BQL	BQL	BQL	BQL	0.102	BQL	BQL	BQL	BQL	BQL	BQL
72	0.433	BQL	(0.0)	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
120	0.069	NA		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pharmacokinetic Parameter														
Cmax (µg/mL or µgEquiv/mL)	20.8	5.23		0.172	0.043	0.269	0.730	1.69	0.437	1.64	0.474	0.072	0.071	1.88
% Cmax for Ranolazine		100		3.29	0.82	5.14	13.9	32.3	8.35	31.4	9.06	1.38	1.36	36.0
Tmax (hr)	0.5	0.5		0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.5	1.0	0.5
AUC $_{(0\text{-}t\ hr\ or\ t)}$	62.9	11.0		0.45	0.12	0.44	1.69	7.77	0.75	3.36	0.86	0.25	0.15	5.14
AUC $_{(0\text{-}24\ hr)}$	86.2	11.2	(13.0)	0.46	0.14	0.47	1.72	12.7	0.76	3.56	0.87	0.26	0.16	5.20
T1/2 (hr)*	31.2	0.7		3.8	2.9	1.0	1.2	6.5	1.1	1.2	1.1	1.8	1.2	1.0
AUC $_{(0-\infty)}$	126	11.0		0.50	0.21	0.48	1.74	13.6	0.76	3.40	0.88	0.36	0.15	6.06
% AUC $_{(0\text{-}24\ hr)}$ for Ranolazine		100		4.07	1.27	4.19	15.3	113	6.73	31.6	7.73	2.33	1.43	46.3

Units for AUC are µg *hr/mL or µgEquiv *hr/mL.
BQL: Below the quantification limit of the assay (<10 ng/mL).
NA: Not analyzed.
*Half-life estimated from last three time points that have measurable levels.

The sponsor states the major pathways of metabolism in the rat as N-dealkylation at both nitrogens of piperazine, amide hydrolysis and O-demethylation of the methoxy group at the methoxyphenyl ring.

Determination of routes and rate of excretion and metabolic profiles of [^{14}C]-ranolazine following oral administration to Beagle dogsCVT303.007-R November16, 2000

Group I consisted of 4 male Beagles and Group II consisted of 3 bile-duct cannulated male Beagles. Dogs were drug-free for at least 3 weeks prior to start of the study. Each dog received a single oral dose of 25 mg/kg aqueous [^{14}C]-ranolazine (~6.25µCi/kg). Blood for determination of plasma radioactivity was collected from all animals pre-treatment, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours post-dose.
Urine was collected: 0-4, 4-8,8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168. Feces were collected pre-dose, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours post-dose. Bile was collected from Group II animals pre-dose, 0-1,1-2, 2-4, 4-6,6-8, 8-10, 10-24, 24-48, 48-72, 72-96, 96-120 hours post dose.
Analysis was by tissue combustion and liquid scintillation counting.

Results: All animals appeared lethargic at 4 hours post-dose but returned to normal appearance by 6 hours. One dog continued to be lethargic at 8 hours after dosing. Maximum concentrations of plasma radioactivity were reported to occur for individuals of both groups from 0.5 – 1.0 hour after dosing. Maximum mean concentrations were observed at 0.5 hours. Mean concentrations decreased to non-detectable levels 4 hours post-dose for Group II and 12 hours post-dose for Group I (vol 43, p.26).

Urinary excretion of radioactivity through 168 hours was 49.29±4.47% of administered dose for Group I animals and 30.28±3.6% for the bile duct cannulated dogs. The majority of radioactivity was excreted within the first 48 hours post-dose for both groups.

Fecal excretion of radioactivity was 42.84±2.13% for the intact dogs and 3.61±0.90% for the cannulated dogs. The majority of excretion was in the first 48 hours. The total amount of radioactivity excreted in the bile (group II) through 120 hours was 59.89±2.70% of the administered dose of radioactivity. Approximately 50% of the administered dose was excreted via the bile within the first 6 hours.
Summary: Consistent with the mouse results, the absorption following oral dosing was rapid with rapid excretion via the urine and bile/feces. Approximately 90% of the dose was excreted within 48 hours for both groups. Greater than 95% was recovered in excreta within 168 hours. Urinary excretion reached 49% in intact animals compared to 30% for the bile duct cannulated animals. This suggests that re-absorbed biliary material contributed to systemic levels in the intact animals. The onset of signs (lethargy) at 4 hours, after the Cmax, may be due either to an active metabolite, delayed distribution of drug to a specific target site or secondary to some pharmacologic process that requires several hours for full effect. See the next report for characterization of metabolites.

Metabolic profiles of ranolazine following oral administration of a single 25 mg/kg dose of [^{14}C]-ranolazine to Beagle dogs.CVT303.002-MET. Amendment to CVT303.007-R August 2002.

The stated purpose of the amendment was to add metabolites that were either not detected or were at concentration that were below the quantification limit of the assay (BQL) in the summary table and footnotes of table 4-21. The various biological matrices that were collected following single oral doses to intact or bile duct cannulated dogs were assessed for metabolites by LC/MS/MS and radiochromatography. Concentrations of 18 metabolites in plasma, urine and bile were quantified by LC/MS/MS. The samples were also subjected to β-glucuronidase/sulfatase hydrolysis to estimate levels of conjugated metabolites.

Results: in the seven days of the study, approximately 49% and 43% of the administered radioactivity was recovered in the urine and feces respectively. At Tmax (0.5 hours), unchanged ranolazine accounted for 44% of the total radioactivity in plasma and decreased to 12% at 8 hours post-dose. Values of plasma AUC_{0-24} of ranolazine accounted for an average of 26% of that of total radioactivity in plasma (p.20, vol 40).

At least 80 metabolites were found in plasma and over 100 were detected in urine. Due to the large numbers of metabolites, complete chromatographic resolution could not be achieved. Of the metabolites that were quantified in plasma, 12 phase I metabolites showed AUC values ≥ 1% of that of ranolazine. The sponsor's findings are shown here.

Metabolites of Ranolazine	Plasma		Urine		Bile
	$AUC_{(0\text{-}24\ hr)}$ (μg .hr/mL) (%Ranolazine AUC)[a]		% Dose		% Dose
Phase I Metabolites	Intact Dogs (n=4)	BDC Dogs (n=3)	Intact Dogs (n=4)	BDC Dogs (n=3)	BDC Dogs (n=3)
Ranolazine (RS-43285, CVT-303)	13.5 (100)	21.1 (100)	2.38	3.55	0.07
RS-88597 (CVT-5030)	6.28 (46.5)	2.30 (10.9)	5.47	2.03	0.00
RS-88640 (CVT-2512)	2.78 (20.6)	0.51 (2.4)	3.91	0.65	0.37
RS-94287 (CVT-2738, Ran 2)	2.79 (20.6)	2.88 (13.6)	2.64	3.04	0.00
CVT-4786	1.92 (14.2)	1.84 (8.7)	3.12	2.69	0.00
RS-89961 (CVT-2551)	1.70 (12.6)	1.80 (8.5)	1.04	0.99	0.02
RS-88681 (CVT-2513)	1.52 (11.2)	1.59 (7.5)	1.52	1.77	0.00
RS-88835 (CVT-5028)	1.00 (7.4)	0.32 (1.5)	0.16	0.00	0.00
RS-88390 (CVT-2514)	0.97 (7.2)	1.00 (4.7)	0.12	0.11	0.00
RS-89289 (CVT-2537)	0.79 (5.8)	0.70 (3.3)	1.83	1.65	0.00
RS-89356 (CVT-5031)	0.60 (4.4)	0.41 (1.9)	0.34	0.19	0.00
RS-88772 (CVT-3388)	0.22 (1.7)	0.16 (0.8)	0.57	0.27	0.00
CVT-2534	0.14 (1.0)	0.20 (0.9)	0.00	0.00	0.00
RS-89983 (CVT-2535)	0.12 (0.9)	0.16 (0.7)	0.00	0.00	0.00
RS-101647 (CVT-3248)	0.0 (0.0)	0.0 (0.0)	0.10	0.10	0.00

Some 10 metabolites had AUC values > 10% that of ranolazine. The sponsor describes these textually as:

Reveiwer's summary of sponsor's textual description of plasma metabolites AUC as % of ranolazine

metabolite	RS-88597	RS-94287	RS-88640	CVT-4786	RS-89961	RS-88681
AUC rel to ranolazine	46.5	20.6	20.6	14.2	12.6	11.2
metabolite	RS88390	RS-88835	RS-89289			
AUC rel to ranolazine	7.2	7.4	5.8			

Data from vol 40., p.20

Pharmacokinetics of Total Radioactivity, Ranolazine, and Metabolites in Intact Dogs Following a Single 25-mg/kg Oral Dose of [^{14}C]-Ranolazine

(Primedica Study BCAZ-101)

(Mean ± SD, N = 4)

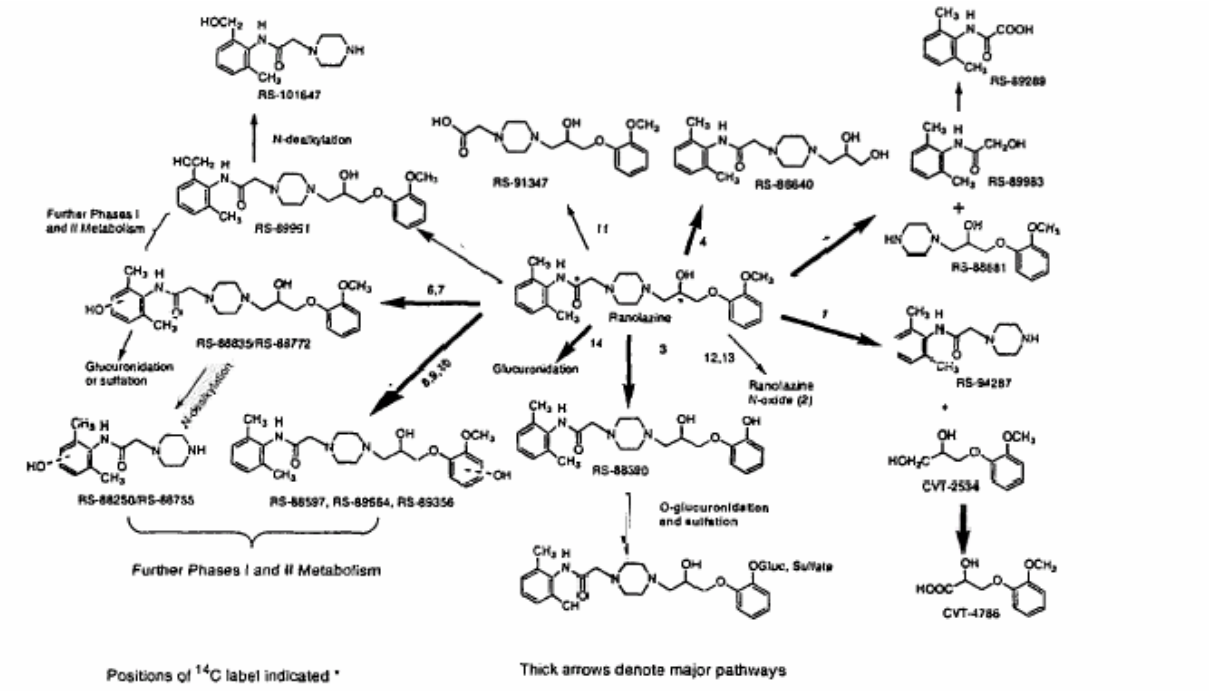
Analyte ID	Cmax (µgEquiv/mL or µg/mL)	Tmax (hr)	$AUC_{(0\text{-}24\ hr)}$ (µgEquiv·hr/mL or µg·hr/mL) (% AUC for Ranolazine)	$AUC_{(0\text{-}inf)}$ (µgEquiv·hr/mL or µg·hr/mL) (% AUC for Ranolazine)	$T_{1/2}$ (hr)
Phase I Metabolites					
Total ^{14}C	16.9 ± 0.89	0.75 ± 0.29	51.2 ± 11.0	51.3 ± 9.73	2.77 ± 0.58
Ranolazine	6.96 ± 1.69	0.63 ± 0.25	13.5 ± 4.91 (100)	13.6 ± 4.99 (100)	4.92 ± 1.54
RS-88597	1.25 ± 0.13	0.75 ± 0.29	6.28 ± 1.58 (46.5)	7.05 ± 2.18 (51.7)	7.49 ± 2.71
RS-94287	0.310 ± 0.170	0.63 ± 0.25	2.79 ± 1.48 (20.6)	3.02 ± 1.43 (22.2)	6.69 ± 2.47
RS-88640	0.179 ± 0.059	1.25 ± 0.50	2.78 ± 0.67 (20.6)	3.90 ± 0.72 (28.6)	13.9 ± 7.02
CVT-4786	1.04 ± 0.63	0.63 ± 0.25	1.92 ± 0.93 (14.2)	1.97 ± 0.87 (14.4)	3.48 ± 1.87
RS-89961	0.363 ± 0.044	0.75 ± 0.29	1.70 ± 0.20 (12.6)	1.70 ± 0.22 (12.4)	3.65 ± 0.97
RS-88681	0.263 ± 0.113	0.63 ± 0.25	1.52 ± 0.70 (11.2)	1.57 ± 0.69 (11.5)	5.25 ± 0.75
RS-88390	0.280 ± 0.035	0.75 ± 0.29	0.97 ± 0.24 (7.2)	1.00 ± 0.30 (7.3)	4.49 ± 1.72
RS-88835	0.324 ± 0.034	0.63 ± 0.25	1.00 ± 0.14 (7.4)	1.17 ± 0.21 (8.6)	9.41 ± 3.74
RS-89289	0.419 ± 0.254	0.63 ± 0.25	0.79 ± 0.47 (5.8)	0.82 ± 0.47 (6.0)	3.69 ± 2.33
RS-89356	0.245 ± 0.039	0.63 ± 0.25	0.60 ± 0.17 (4.4)	0.63 ± 0.18 (4.6)	4.87 ± 2.19
RS-88772	0.074 ± 0.011	0.75 ± 0.29	0.22 ± 0.05 (1.7)	0.25 ± 0.05 (1.8)	2.64 ± 0.80
CVT-2534	0.101 ± 0.074	0.63 ± 0.25	0.14 ± 0.10 (1.0)	0.15 ± 0.10 (1.1)	0.69 ± 0.18
RS-89983	0.087 ± 0.049	0.63 ± 0.25	0.12 ± 0.07 (0.9)	0.12 ± 0.07 (0.9)	0.73 ± 0.11
Phase II Metabolites[a]					
RS-88597 Conjugate[b]	1.29 ± 0.24	1.50 ± 0.58	8.70 ± 2.32 (64.4)	10.1 ± 2.99 (73.7)	8.53 ± 2.51
Ranolazine Glucuronide	2.65 ± 1.00	1.00 ± 0.00	8.13 ± 2.97 (60.1)	8.11 ± 2.92 (59.4)	3.78 ± 0.83
RS-88835 Conjugate	1.04 ± 0.289	0.75 ± 0.29	4.96 ± 1.50 (36.7)	5.99 ± 1.87 (43.9)	10.0 ± 4.1
RS-89356 Conjugate	0.435 ± 0.095	1.25 ± 0.50	2.81 ± 0.77 (20.8)	3.19 ± 0.86 (23.4)	7.81 ± 2.72
RS-88390 Conjugate	0.272 ± 0.073	1.13 ± 0.63	2.16 ± 0.39 (16.0)	2.72 ± 0.73 (19.9)	10.4 ± 6.8
RS-88640 Conjugate	0.076 ± 0.025	1.75 ± 0.50	0.46 ± 0.18 (3.4)	0.63 ± 0.39 (4.6)	9.93 ± 10.9
RS-89983 Conjugate	0.059 ± 0.023	1.33 ± 0.58 (n=3)	0.15 ± 0.11 (1.1)(n=3)	1.52 (n=2)	

[a] Conjugates of RS-89664, RS-89961, RS-88772, CVT-2534, desmethyl RS-88681, and RS-88755 were either not detected or levels were BQL.

[b] Concentrations of conjugates were estimated from the differences before and after ß-glucuronidase hydrolysis.
The hydrolying enzyme used contained both ß-glucuronidase and sulfatase.

Metabolites of Ranolazine	Plasma		Urine		Bile
	$AUC_{(0\text{-}24\ hr)}$ (μg .hr/mL) (%Ranolazine AUC)[a]		% Dose		% Dose
Phase II Metabolite[b]	Intact Dogs (n=4)	BDC Dogs[c] (n=3)	Intact Dogs (n=4)	BDC Dogs (n=3)	BDC Dogs (n=3)
RS-88597 (CVT-5030) Conjugate	8.70 (64.4)	3.05 (14.4)	4.01	1.48	6.29
Ranolazine Glucuronide	8.13 (60.1)	6.12 (29.0)	4.32	3.71	33.41
RS-88835 (CVT-5028) Conjugate	4.96 (36.7)	1.16 (5.5)	1.62	0.22	4.67
RS-89356 (CVT-5031) Conjugate	2.81 (20.8)	0.79 (3.7)	2.75	1.04	5.03
RS-88390 (CVT-2514) Conjugate	2.16 (16.0)	1.02 (0.48)	0.90	0.35	3.48
RS-88640 (CVT-2512) Glucuronide	0.46 (3.4)	0.47 (2.2)	0.57	0.20	0.48
RS-89983 (CVT-2535) Conjugate	0.15 (1.1)	0.00 (0.0)	0.15	0.11	0.00
RS-89961 (CVT-2551) Conjugate	0.00 (0.0)	0.00 (0.0)	0.33	0.27	0.57
RS-88772 (CVT-3388) Conjugate	0.00 (0.0)	0.00 (0.0)	0.10	0.09	0.15
CVT-2534 Conjugate	0.00 (0.0)	0.00 (0.0)	0.28	0.24	0.00

[a]Values represent % AUC of ranolazine based on μg.hr/mL. Ranolazine plasma AUC was set at 100%.
[b]Units for conjugates are expressed as μg of the corresponding Phase I metabolite equivalent.hr/mL.
The following metabolites were either not detected or at levels that were below the quantification limit of the assay: RS-91347 (CVT-3369) and conjugates of RS-89664 (CVT-5029), desmethyl RS-88681 (CVT-3247) and RS-88755 (CVT-3389).



The sponsor reports that the metabolic profile of ranolazine in dogs was qualitatively similar to humans. Quantitative differences were noted. For example, direct glucuronidation of ranolazine and hydroxylation of the methoxyphenyl and dimethylphenyl rings were major routes of metabolism in the dog and minor in humans. O-demethylation followed by sulfation and glucuronidation were major in humans but minor in dogs.

A major new metabolite identified in the current study was CVT4786, the carboxylic acid derivative of CVT-2534. Other newly identified metabolites were two N-oxides at the N1 and N4 piperazine positions.
Other metabolites that the sponsor felt worthy of special mention were:
N-dealkylation (at the N1 position of piperazine ring):
RS-88681 and RS-89983 (further oxidized to RS-89289)
O-dearylation- RS-88640
O-demethylation- RS-88390
Amide hydrolysis- RS-91347 and

Glucuronidation, N-dealkylation, O-demethylation and hydroxylation appear to be main metabolic routes in the dog.

The sponsor reports that there were no metabolites unique to plasma. However, endogenous compounds may mask the identification of products of drug metabolism. The reviewer is unaware of any studies examining the presence of interfering endogenous substances for any of the preclinical species.

Excretion studies in mice in support of a three month carcinogenicity dose ranging study with ranolazine (RS-43285 VHT(2)) AT4945, October 1989
Satellite groups of mice were used for this study. Male and female mice in the satellite groups received the LD (5 mg/kg/day) and HD (35 mg/kg/day) from the 3 month study. A second satellite group was used for a single dose excretion study. These male mice received a single oral administration of 5 mg/kg ^{14}C-ranolazine (labelled on the carbonyl carbon of the acetamide group). Urine and feces were collected daily. Metabolism cages were washed at the end of the observation period to account for all possible radioactivity. Radioactivity was analyzed by liquid scintillation counting.

Results: There were essentially no differences between the single dose results and those who received multiple doses. The results are summarized in the reviewer's table below.

Reviewer's Summary of Mean Excretion of Radioactivity (p. 288)

Parameter	5 mg/kg ^{14}C-ranolazine		
	Single dose males (n=6)	Multi-dose males (n=6)	Multi-dose females (n=6)
Urinary excretion	51±4	55±7	54±7
Fecal excretion	41±4	39±6	40±9
Total excretion	91±6	94±3	95±6
35 mg/kg ^{14}C-ranolazine			
Urinary excretion		61±7	60±5
Fecal excretion	Not reported	35±6	36±4
Total excretion	Not reported	96±2	96±2

Values are mean % dose recovered ±SD

Approximately 90% of dosed radioactivity was recovered within 24 hours.
There was minimal recovery of radioactivity from the carcass and GI contents under all conditions. A slight increase in urinary pH was reported for the multiple dose animals of both sexes compared to the single dose animals where there was no reported change in urinary pH. The LD animals went from pH 7.1-pH 8.0. The HD mice went from pH 7.1 to pH 7.9. The significance of this is not clear.

Plasma level and excretion studies in mouse following multiple oral administration of ranolazine in support of the two year carcinogenicity study (RS-43285 VMT) AT6153, January 1989-January 1991. Report: July 1992.

A satellite group of thirty male and 30 female VAF CD1 mice were assigned to each of two dose groups. Ranolazine was given at dose levels of 5 or 50 mg/kg/day for the duration of the study. Blood samples were collected at 30 minutes post-dose from up to 10 animals per sex group on day 1 and at 3,6,12,18 and 24 months of the study. Plasma was examined for ranolazine concentration. On selected days at approximately 3 and 9 months, the usual daily dose was replaced by a ^{14}C-ranolazine dose. On these occasions, male and female mice (n=4 or 6 from each dose group) were housed in polycarbonate metabolism cages until 96 or 120 hours post-dose and excreta collected daily. Urine and feces were assayed for radioactivity at the 3 month timepoint. Urine only was assayed for radioactivity at the 9 month timepoint. HPLC detection was conducted.

Results: Ranolazine was found in the plasma at each sampling period for both doses. There was insufficient data generated for AUC determination. The differences between the plasma levels at single timepoint determinations really can't be given the same level of comparison as AUC data.

For all groups, excretion of radioactivity was primarily through the urine. The percent dose recovered was from 47%-58% for both sexes, all dose groups at both 3 and 9 months. "Representative" urinary metabolite profiles were presented as chromatograms with minimal information.

A preliminary investigation of the pharmacokinetics of ranolazine in mouse following single oral and chronic oral administration AT6291, January 1993.
In the preliminary study, male mice were given single oral doses of ^{14}C-ranolazine at 15 and 35 mg/kg. Terminal blood samples from 3 animals per time point (20 min., 40 min., 1, 1.5, 2,4,6,8 and 24 hours post-dose) were analyzed for ranolazine and radioactivity. An additional study was undertaken in 3 mice to examine earlier timepoints of 5,10,15,20, 25 and 30 minutes post-dose. In the carcinogenicity dose ranging study, there were two active satellite treatment groups with 20 male and 20 female mice in each dose group. The dose levels were 5 and 35 mg/kg/day. Blood samples were taken from each mouse at 30 minutes post-dose on Days 1, 8, 28 and 84 of the study. Plasma samples were analyzed for ranolazine.

Results: In the preliminary study, exposure increased more than proportionally with increasing dose as seen in both Cmax and AUC. This non-proportional increase was seen in both ranolazine and drug-derived radioactivity. This is summarized in the sponsor's table below. It is not clear if the extra mice used for the early timepoints were included in these calculations.

Table 4
MEAN C_{max}, t_{max} and $AUC_{0-\infty}$ VALUES FOLLOWING SINGLE ORAL ADMINISTRATION OF ^{14}C-RANOLAZINE AT 15 AND 35 mg/kg TO MALE MICE

	Dose	
	15 mg/kg	35 mg/kg
Ranolazine		
C_{max} (ng/ml)	1480	4670
t_{max} (h)	0.333	0.333
$AUC_{0-\infty}$ (ng.h/ml)	2920	9100
Radioactivity		
C_{max} (ng/ml)	4790	11200
t_{max} (h)	1.00	0.333
$AUC_{0-\infty}$ (ng.h/ml)	13200	34100

In the second study, where a single sample was taken at 30 minutes after dosing, The mean plasma level of ranolazine remained relatively constant over the duration of the study. This is summarized in the reviewer's table below.

Reviewer's summary of mean plasma ranolazine at 30 minutes post dose (units not given in report pp. 84-87)

	Day 1	Day8	Day 28	Day 84

Females 5 mg/kg/day	253±89.7	189±56.8	201±59.1	176±54.4
Males 5 mg/kg/day	224±79.8	244±151	263±87.5	242±99.5
Females 35 mg/kg/day	2350±909	2300±775	2900±860	2990±1530
Males 35 mg/kg/day	2960±808	3230±928	2620±691	3500±1480

Metabolic profiles of ranolazine following oral administration of a single 50-mg/kg dose of [^{14}C]-ranolazine to male mice. CVT303.006-MET, July 30, 2002

Male CD-1 mice received single oral doses of [^{14}C]-ranolazine at 50 mg/kg. Concentrations of total radioactivity in plasma and recoveries of the radioactive dose in urine and feces up to 5 days post-dose were determined. Plasma and urine samples were analyzed by LC/MS/MS. The samples were also subjected to β-glucuronidase/sulfatase hydrolysis to estimate levels of conjugated metabolites. The report does not state the number of mice used, how many were sampled per time point and the times at which plasma samples were collected. Urine samples were apparently collected over 5 days with 0-48 hour samples pooled "proportionally to the total volume to make one urine pool for each mouse."

Results
Urine: Nine major metabolites were detected by radiochromatography of the 0-48 hour pooled sample. Fifty-nine additional minor metabolites were also detected by both radiochemical detection and MS.

Plasma: the sponsor states that most urinary metabolites were also detected in plasma with no metabolites that were unique to plasma. However, the sponsor adds a caveat that the presence of major novel metabolites may have been masked by significant chemical noise from endogenous compounds in the total ion chromatogram generated by LC/MS. It is stated in the report that concentrations of 18 Phase I and 13 Phase II metabolites were determined. It does not state how many metabolites overall were identified or counted. Apparently there were sufficient numbers of metabolites that complete resolution could not be achieved. The greatest exposure based on AUC was to ranolazine (18% of total ^{14}C) followed by RS-94287 (6%), RS-88390 conjugate (7%), RS-88681 conjugate (5%), RS88597(5%) and RS-89983(4%).

The sponsor's summary is shown below.

Metabolites of Ranolazine RS Number (CVT Number)	Plasma $AUC_{0\text{-}24\ hr}$ (µg x hr/mL) (% AUC for Ranolazine)	Urine % Urinary Total Radioactivity (% of Administered Dose)
Phase I Metabolites		
Ranolazine (RS-43285, CVT-303)	8.88 (100)	2.44 (1.18))
RS-94287 (CVT-2738, Ran 2)	2.88 (32.4)	13.7 (6.61)
RS-88597 (CVT-5030)	2.25 (25.3)	1.16 (0.56)
RS-89983 (CVT-2535)	2.04 (23.0)	0.88 (0.43)
CVT-4786 (Acid of CVT-2534)	1.31 (14.7)	18.7 (9.05)
RS-88681 (CVT-2513)	1.22 (13.7)	10.5 (5.07)
RS-89289 (CVT-2537)	0.93 (10.5)	13.9 (6.73)
RS-89961 (CVT-2551)	0.88 (9.90)	2.22 (1.07)
RS-91347 (CVT-3369)	0.77 (8.69)	0.98 (0.47)
CVT-2534	0.23 (2.56)	0.00 (0.00)
RS-101647 (CVT-3248)	0.14 (1.55)	0.94 (0.46)
RS-88390 (CVT-2514)	0.12 (1.37)	0.00 (0.00)
RS-88755 (CVT-3389)	0.12 (1.30)	0.67 (0.33)
RS-89356 (CVT-5031)	0.10 (1.15)	0.00 (0.00)
Desmethyl-RS-88681 (CVT-3247)	0.06 (0.64)	0.74 (0.36)
RS-88250	0.03 (0.32)	0.55 (0.27)
RS-88640 (CVT-2512)	0.011 (0.12)	0.97 (0.47)
RS-88772 (CVT-3388)	0.012 (0.14)	0.00 (0.00)
RS-88835 (CVT-5028)	0.008 (0.08)	0.00 (0.00)
Phase II Metabolite[b]		
RS-88390 (CVT-2514) Conjugate	3.16 (35.6)	5.37 (2.62)
Desmethyl-RS-88681 (CVT-3247) Conjugate	2.54 (28.6)	3.30 (1.59)
RS-89983 (CVT-2535) Conjugate	2.06 (23.2)	6.12 (2.95)
RS-88597 (CVT-5030) Conjugate	1.98 (22.3)	3.68 (1.79)
Ranolazine (RS-43285 or CVT-303) Glucuronide	1.32 (14.9)	0.71 (0.35)
RS-88835 (CVT-5028) Conjugate	0.42 (4.76)	0.95 (0.46)
RS-88250 Conjugate	0.40 (4.53)	0.30 (0.15)
RS-89356 (CVT-5031) Conjugate	0.36 (4.10)	1.19 (0.58)
RS-88772 (CVT-3388) Conjugate	0.30 (3.42)	0.61 (0.30)
RS-88755 (CVT-3389) Conjugate	0.28 (3.15)	0.37 (0.18)
RS-88640 (CVT-2512) Conjugate	0.22 (2.52)	0.00 (0.00)
RS-89961 (CVT-2551) Conjugate	0.18 (2.00)	0.00 (0.00)
CVT-2534 Conjugate	0.11 (1.21)	0.65 (0.31)

[a]Values represent % AUC of ranolazine. Ranolazine plasma AUC was set at 100%.
[b]Units for conjugates are expressed as µg of the corresponding Phase I metabolite equivalent.hr/mL.

Results of this study are consistent with previous reports that ranolazine is extensively metabolized following oral administration in the mouse. In addition to the metabolites already identified in previous studies, several new metabolites were found. These included CVT-4786, which was the oxidative product of CVT-2534, the complimentary half of RS-94287. N-oxides of ranolazine, several di- and tri-hydroxylated ranolazine and RS-88390 derivatives and their methylated metabolites.

It may be concluded that ranolazine is extensively metabolized in male mice. Several previously unreported metabolites were identified. These included CVT-4786 and 2 N-oxides at the N1 and N4 piperazine positions. There were other new, methylated metabolites derived from dihydroxylated ranolazine. One metabolite found in human plasma but not in the mouse is a conjugate of RS-89664, a hydroxylated metabolite at the 3-position of the methoxyphenyl ring.

There are quantitative differences in the human vs mouse metabolite profiles. N1-dealkylation was a major route of metabolism in mice but was minor in humans. O-demethylation and O-dearylation were significant metabolic routes in humans but not in mice.

Plasma level and excretion studies in the male dog following single oral administration of ^{14}C-ranolazine at 60 mg/kg AT5957 Conducted Oct. 1986, Reported: January 1992.

Ranolazine (lot E6-ML-001) and ranolazine labelled with ^{14}C on the carbonyl carbon atom of the thioacetamide group (specific activity 0.74 MBq/mg) was used. Three male Beagles (Alpha Sirius, UK) were given aqueous solutions of drug in a single oral dose of 60 mg/kg. The dose level used in this study matched the dose used in the 6-month dog oral toxicity study. Urine and fecal samples were collected each day for 7 days.
Plasma levels of drug were determined by HPLC methods. The report does not specify when the blood samples were collected, but a page in the results section suggests that the times were 0.25, 0.5, 0.75, 1 1.5, 2, 3, 4, 6, 8, 12, 24 ,30, 48 and 72 hours after dosing.

Results: Drug derived radioactivity persisted in the plasma for a longer time than did the parent drug. Bioavailability of the parent drug was high, on average 65±15%. The sponsor's results are shown at right. The mean tmax values were 1.9 and 2.5 hours for ranolazine and radioactivity respectively. Both Cmax and AUC for radioactivity were higher than the respective values for ranolazine.

Pharmacokinetics of Ranolazine and Radioactivity in the Male Dog Following Single Oral Administration of ^{14}C-Ranolazine (60 mg/kg)

Parameter	Dog No. M4CA1	M4CA2	M4CA5	Mean ± SD
Radioactivity				
C_{max} (ng equiv/ml)	12800	8140	7650	9530 ± 2840
t_{max} (h)	2.0	1.5	4.0	2.5 ± 1.3
AUC(0-infinity) (ng equiv.h/ml)	142000	102000	85200	110000 ± 29200
Terminal $t_{1/2}$ (h)	17.4	20.0	14.6	17.3 ± 2.7
Ranolazine				
C_{max} (ng/ml)	2140	2310	2150	2200 ± 95.4
t_{max} (h)	0.8	1.0	4.0	1.9 ± 1.8
AUC(0-infinity) (ng.h/ml)	11900	12100	11500	11800 ± 306
Terminal $t_{1/2}$ (h)	5.60	4.10	10.9	6.87 ± 3.57
Oral Clearance (ml/min/kg)	84.0	82.6	87.0	84.5 ± 2.25
Bioavailability** (%)	78.7	49.3	67.9	65.3 ± 14.9

^{14}C AUC (po, 5 mg/kg, 0-inf) = 11200 ± 2600 ng equiv.h/ml*
^{14}C AUC (iv, 5 mg/kg, 0-inf) = 10700 ± 1900 ng equiv.h/ml*
* data reproduced from reference 1
** determined using individual systemic clearance values following a 5 mg/kg iv dose (see Appendix A)

The sponsor compared the results from the current 60 mg/kg oral dose with previous studies of 5 mg/kg IV and po in the dog. In the sponsor's results below, it may be seen that there is a less than proportional increase in Cmax after the 5 and 60 mg/kg doses and a more than proportional increase in AUC $_{0-\infty}$. The clearance dropped by approximately 50% at the higher dose while the Vd increased. Mean urinary excretion of radioactivity was 32% and mean fecal excretion was 68%. This is the reverse of what has been

reported for most species (predominantly urinary excretion).

Summary of Pharmacokinetic Parameters of Ranolazine in Dog Following Single Intravenous and Single Oral Administration of ^{14}C-Ranolazine

Parameter	5 mg/kg iv* (n=4)	Parameter	5 mg/kg oral* (n=4)	60 mg/kg oral ** (n=3)
C_0 (ng/ml)	4660 ± 983	C_{max} (ng/ml)	265 ± 128	2200 ± 95.4
		t_{max} (h)	0.4 ± 0.2	1.9 ± 1.8
Terminal $t_{1/2}$ (h)	0.56 ± 0.48	Terminal $t_{1/2}$ (h)	1.01 ± 0.55	5.87 ± 3.57
$AUC_{0-infinity}$ (ng.h/ml)	1650 ± 373	$AUC_{0-infinity}$ (ng.h/ml)	471 ± 116#	11800 ± 306
Systemic Clearance (ml/min/kg)	52.6 ± 12.0	Oral Clearance (ml/min/kg)	185 ± 50.0#	84.5 ± 2.25
Volume of Distribution (L/kg)	2.27 ± 1.50	Bioavailability+ (%)	27.6 ± 9.47#	65.3 ± 14.9

* Parameter values calculated from data in reference AT 3407 (SS/038/85). Individual values are given in Appendix A of this report (SS/039/89)
* 60 mg/kg data from this report (SS/039/89)
# n=3
+ Bioavailability values calculated using individual systemic clearance values.

The study reports increased oral bioavailability (from 28% at 5 mg/kg to 65% at 60 mg/kg), decreased clearance, increased volume of distribution and non-linear increases in exposure with increasing oral dose. This is suggestive of saturated clearance, evidenced by a terminal $t_{1/2}$ of 1 hour at 5 mg/kg and 7 hours at 60 mg/kg, or tissue storage. The more rapid loss of parent compound compared to overall drug-derived radioactivity suggests metabolism and, if the pharmacologic effect persists past the disappearance of parent drug, active metabolites.

Other studies:

Summary of the disposition and metabolic effects of RS 43285-193 in animals. AT3840 March 1987. RS 43285 was radiolabeled with ^{14}C on the carbonyl carbon of the acetamide group for these studies.

This is the sponsor's summary of the above metabolism studies. No new information was presented. It was noted that the mean oral AUC was 20% of the IV value. Is this consistent with the levels of radioactivity absorbed in the oral dose studies?

The effect of ranolazine on rat liver cholesterol 7 alpha-hydroxylase activity. AT4961, November 1989

The report notes in the introduction that the study was prompted by the adrenal pathology findings in the 3 month rat study.

Male Sprague-Dawley rats (Charles River, UK) were euthanized and the livers collected. Microsomes were then prepared and the microsomal protein content determined. Hepatic microsomal 7αhydroxylase activity was measured by isotope incorporation. Concentrations of

ranolazine of 10^{-9}, 10^{-8}, 10^{-7},10^{-6},10^{-5}M were tested for their effect on the enzyme. The effect of metyrapone at 10^{-4}M was used as a positive control.

Results: The positive control caused a 26% decrease in enzymatic activity (0.84±0.06 pmol/min/mg protein for the control vs 0.69±0.14 pmol/min/mg/protein for metyrapone). No consistent effect was discernible for the test compound. One wonders why, for purposes of identification of effect, that the test compound was not tested at the same or a higher concentration than the positive control.

An in vitro investigation of the potential invovlement of cytochrome P450 3A in the hepatic metabolism of ranolazine. CL6906/ SS/035/94 December, 1994

Rat liver microsome from control and dexamethasone-treated male CD/SD rats were prepared previously according to a referenced study. Human liver was obtained frozen from a tissue bank (Keystone Skin Bank, Exton, USA). In vitro rate incubations were carried out using ranolazine in water at concentrations from 0.1- 10 µM. Other liver microsome samples were used to try to correlate rate of ranolazine metabolism with CYP3A activity. Testosterone hydroxylase activity was determined by measuring the rates of formation of 2β-, 6β- and 7α-hydroxytestosterone and 4-androsten-3,17-dione. Incubations in the presence of ketaconazole and triacetyloleandomycin (TAO) were performed in a similar manner to the in vitro rate incubations. Ketaconazole and TAO were added to the reaction mixtures at final concentrations of 0.05-100µM and 10-100 µM respectively. It appears from the methods section, that replicates were not uniformly used. An incubation was conducted in a similar manner to the in vitro rate incubations in the presence of TAO and sulphaphenazole at final concentrations of 10 and 50 µM respectively. The reaction proceeded for 30 minutes followed by the addition of ranolazine. The reaction then continued for another 60 minutes. Incubations were also conducted in a similar manner to the in vitro incubations in the presence of untreated and dexamethasone-treated rat liver microsomes at final concentrations of 10µM ranolazine. The reaction was allowed to proceed for 10 minutes. Samples were analyzed by HPLC for ranolazine and metabolite peaks. MS and LC-MS analysis were also employed.

MASS SPECTRAL ANALYSIS OF RANOLAZINE METABOLITES FROM DEXAMETHASONE-TREATED RAT LIVER MICROSOMES AND TAO-INHIBITED HUMAN LIVER MICROSOMES

Ranolazine Metabolite (RS-)	Dexamethasone-Treated Rat Microsomes*	TAO-Inhibited Human Microsomes*	Sulphaphenazole-Inhibited Human Microsomes*
89983	↑	ND	ND
94287	↑↑	↓	↑
88755	↑↑	ND	↑
88681	↑	↓	↑
88640	↑↑	ND	↑
91347	↑	ND	=
88390	↓	=	↑
88772	↑	↓	=
88597	=	=	=
88835	↑	↓	=
89961	↑	↓	=

* Arrows indicate level changes relative to ranolazine level in the sample.
↑ increased level in relation to unchanged ranolazine
↓ decrease level in relation to unchanged ranolazine
= equal level compared to control

ND not detected in human liver microsomes

Results: The presentation of the data is suboptimal. Rate constants were provided for the in vitro rate of metabolism incubations in untreated human liver microsomes, in control and dexamethasone-induced rat liver microsomes, in the presence of ketaconazole and TAO. The values are based on single incubations. A dose-dependent increase in inhibition of ranolazine metabolism was seen with increasing concentrations of ketaconazole. Concentrations of 10 and

100 μM ketaconazole produced ~ 82-83% inhibition of ranolazine metabolism. Concentrations of 10 and 100 μM TAO produced 63% and 82% inhibition of ranolazine metabolism also. Both of these inhibitors were tested without replicates. The mass spectral analysis of ranolazine metabolites is shown here.

It was stated in the text of the report that levels of RS-88390 (O-desmethyl metabolite) and RS-88597 (hydroxylated metabolite) "appeared neither to increase in the CYP3A-induced microsomes nor decrease in the CYP3A-inhibited microsomes." This cannot be evaluated in the data as presented.

Distribution of ranolazine to human blood cells and binding of ranolazine to human plasma, human serum albumin, and human α-1 glycoprotein in vitro by an ultrafiltration method. CVT303.001-N August, 1998.

Human blood was collected from 3 healthy male volunteers. Citrated plasma was collected and used the same day as blood sampling. Purified human serum albumin and α-1acid glycoprotein were purchased from Roche Bioscience. Plasma, human serum albumin or α-1acid glycoprotein was mixed with a fixed amount of [^{14}C]-ranolazine and varying concentrations of unlabelled ranolazine then incubated at 37° for 3 hours. Ultrafiltration was performed on the equilibrated, room temperature incubation systems. Blood (presumably whole) was mixed with [^{14}C] ranolazine and varying concentrations of unlabeled ranolazine and incubated at 37°C for 20 minutes. The blood samples were then centrifuged to separate plasma from rbcs. Samples were analyzed by liquid scintillation counting and/or liquid chromatography.

Results A very small range (0.25 to 10 μg/ml) of ranolazine concentrations was tested. It does not appear that concentrations of protein were varied. It appears from the results that approximately 60-64% of the drug-derived radioactivity is bound in human plasma. An n=1 for α-1 acid glycoprotein binding is presented with insufficient labeling to permit accurate interpretation. The mean percent bound (dpm/ml?) ranges from 47-62% for ranolazine concentrations of 10μg/ml to 0.25μg/ml respectively. The mean percent bound (dpm/ml?) to human serum albumin is reported as approximately 30%. The concentration of ranolazine in blood: concentration in plasma was less than 1 for all 3 volunteers.
Summary: It appears that ranolazine is moderately protein bound and does not sequester in rbcs.

Binding of ranolazine to mouse, rat and dog plasma in vitro by an ultrafiltration method. July 29-Aug.3, 2001; Reported June , 2002. CVT303.019-N
Binding of ranolazine to plasma from mouse, rat and dog was determined in vitro by ultrafiltration methods. [^{14}C]-ranolazine at concentrations of 0.5 -10 μg/ml was incubated with pooled plasma from male CD-1 mice (n=20), male Sprague-Dawley rats (n=6) or male Beagles (n=3) at 37ºC for ≥0.5 hours. The animals were drug-free for at least one week prior to blood collection. The unbound fraction in the plasma was separated by centrifugation through a membrane. The radioactivity in plasma and filtrate was determined by liquid scintillation counting.

Results

Mouse: There was no difference in % bound across 0.5 – 10 μg/ml (58.9±0.710 – 57.0±0.445).
Rat: There was no difference in % bound across 0.5 – 30 μg/ml (56.2±0.310 – 53.3±0.971)
Dog: there was no difference in percent bound across 0.5 – 30 μg/ml (47.1±0.580 – 43.7±0.713).
By comparison, binding of ranolazine in human plasma was 60.9-63.9% over the concentration range of 0.25-10 μg/ml.
Ranolzine is moderately plasma protein bound when tested in vitro in the species studied.

PK/TK summary: The studies would be stronger if data was presented to show that there were no endogenous interfering substances in any of the biological matrices.

Ranolazine was rapidly absorbed after oral administration in all non-clinical species. The drug was widely distributed, extensively metabolized and cleared almost equally via urine and feces.

Absorption
Absorption after oral dosing was fairly rapid, with the species studied showing Tmax within 0.5-2 hours.

Distribution
Whole body autoradiography studies following single oral doses, some studies including quantitation by liquid scintillation counting, showed principal tissue levels of radioactivity at 1 hour in the GI tract, liver, adrenals, kidney, thyroid, arterial wall, bone marrow, heart and brain. At 72 hours post-dose, radioactivity was primarily associated with liver, kidney, GI tract and thyroid, although detectable levels were reported for all the original tissues. Single oral doses of ranolazine given to pigmented rats showed that the eyes retained measurable levels of radioactivity. Albino rats showed detectable levels of radioactivity in the eyes (~0.35% x $10^{-3}$of the administered dose) that were no longer detectable after 2 days. The elimination half-life for the pigmented eyes was 23 days.

Metabolism

A small percentage of ranolazine appears to be excreted unchanged. The remainder is highly metabolized by CYP450 3A4, CYP450 2D6 and Phase II conjugations. Approximately 40 metabolites were reported in the plasma of rats and more than 80 metabolites found in the urine. Similar findings were reported for dogs. Incomplete resolution of peaks makes it possible that more metabolites exist. There are at least 12 metabolites that regularly appear across species at levels greater than 1% of the AUC for ranolazine. Qualitatively these metabolites are those reported for human plasma samples. Following the metabolites across all studies becomes somewhat challenging as the codes used changed over the course of the studies. The metabolites of interest are shown.

Table 5.5-11 Comparison of Systemic Exposure to Metabolites of Ranolazine in Plasma (AUC) Following a Single Oral Dose of [Carbonyl, Propyl-2 ^{14}C]-Ranolazine in Male CD-1 Mice, Sprague Dawley Rats, Beagle Dogs, and Humans (Cont'd)

	Plasma AUC (µg.h/mL)			
Species	Mouse	Rat	Dog	Human
Reference	CVT303.006-MET	CVT303.003-MET	CVT303.002-MET	CVT303.001-MET
Dose (mg/kg)	50	50	25	500[a]
Ranolazine	8.88	11.2	13.5	7.67
Phase II Metabolites[b,d]				
RS-88390	**3.16**	**3.79**	**2.16**	**4.55**
Ranolazine[d]	**1.32**	**1.08**	**8.13**	**1.46**
RS-88597	**1.98**	**1.37**	**8.70**	**1.02**
RS-89664	0.00	0.00	0.00	0.49
RS-89356	0.36	0.22	**2.81**	0.36
RS-89961	0.18	0.00	0.00	0.35
RS-88835	0.42	0.16	**4.96**	0.27
RS-88640	0.22	0.00	0.46	0.00
RS-88772	0.30	0.00	0.00	0.00
CVT-2534	0.11	0.14	0.00	0.00
Desmethyl RS-88681	**2.54**	0.00	0.00	0.00
RS-89983	**2.06**	0.00	0.15	0.00
RS-88250	0.40	NA[c]	NA	NA
RS-88755	0.28	0.00	0.00	0.00

[a] Humans received a dose of 500 mg ranolazine solution, pH 4.
[b] Bolded values represent those with plasma AUC > 1 µg.h/mL
[c] NA = Not analyzed
[d] All Phase II metabolites were a mixture of glucuronides and sulfates, except for ranolazine, which consisted of only glucuronides.

In one dog study, an oral dose of 25 mg/kg aqueous ranolazine produced signs of lethargy from 4 to 8 hours post-dose. The findings are suggestive of an active metabolite.

In reports CVT303.006.MET (July 2002, mice),CVT303.003.MET amendment dated August 2002 (rats) and CVT303.002.MET (dog) the sponsor cites CVT4786 as a major new metabolite recently identified. In the rat study this accounted for 9.4% of the total radioactivity in urine (parent drug accounted for only 4% of the urinary radioactivity). In the dog study, CVT4786 had an AUC of 14.2% relative to ranolazine.

Excretion

The majority (94-98%) of the radiolabelled material was excreted within the first 48 hours after dosing. The routes of excretion tended to be divided equally between urinary and fecal.

Protein Binding

It appears that 60-64% of drug-derived radioactivity is protein bound in human plasma. Plasma protein binding was slightly lower in the non-clinical species. The average levels in mice, rats and dogs were ~58, 55 and 45% respectively. The data also indicate that the drug does not sequester in rbcs.

PK/TK conclusions: The sponsor detected the drug and derived material, however, the above studies would be stronger for the demonstration of limits of detection and limits of quantitation. The studies would be further strengthened by demonstration that the biological matrices of each species held no endogenous substances that interfered with detection/quantitation. Many of the studies contained small sample sizes and/or incomplete reporting of methodology. Despite these limitations, one comes to the conclusion that the drug was absorbed relatively quickly, with an oral bioavailability that increased from 28% at 5 mg/kg to 65% at 60 mg/kg. The drug is highly metabolized by both Phase I and Phase II routes in all species. CYP3A4 and CYP2D6 have been identified as involved. Major

routes of proposed metabolism include N-dealkylation at both nitrogens of the piperazine, amide hydrolysis and O-demethylation of the methoxy group at the methoxyphenyl ring. O-demethylation followed by glucuronidation and sulfation were reportedly major in humans but minor in dogs. The non-clinical species produce at least a qualitative representation of the major metabolites found in humans. Excretion appears to be essentially complete within approximately 120 hours after ingestion. The majority of drug is excreted within the first 48 hours, divided relatively evenly between urinary and fecal routes.

Other limitations of the characterization of metabolism include lack of plasma level data for the reproductive and developmental toxicology studies. There is also no distribution data for these studies.

IV. GENERAL TOXICOLOGY:

Study title:RS 43285 RBT: Oral EMLD study in rats

Key study findings: A single oral dose of 250 mg/kg produced 40% mortality while a dose of 500 mg/kg produced 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis.

Study no: AT3414, SS/051/85
Volume #, and page #: vol 16, p.6
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no ( )
Drug, lot #, radiolabel, and % purity: lot # 125SS0584, 100%x
Formulation/vehicle:

Methods The purpose of the study was to estimate the mean lethal oral dose. Fasted Sprague-Dawley rats (Charles River, Kent) 5 males and 5 females per group were given a single oral gavage dose of either 250 or 500 mg/kg of ranolazine. Survivors were euthanized after 14 days with no histopathological assessments.

Results: At the HD, 1/5 m and 2/5f were found dead within 12 minutes of dosing. Clinical signs were seen 2-5 minutes after dosing and included prostration, dyspnea, convulsions, pale extremities (due to vasoconstriction, an observation that was not explained) and salivation.

The animals in both dose groups showed signs within 30 minutes of dosing that included subdued behavior, prostration, convulsions, hyperventilation, piloerection, hunched appearance, ptosis and salivation (HD: 2/5 m, 1/5f; LD: 1/5 m and 3/5 f). No improvement was noted after 2.5 hours, thus the animals were euthanized for humane reasons. It should be noted that in a previous study, animals were dosed at the same levels for a period of three months. The sponsor suggests that fasting the animals in the current study was in part responsible for the poor survival.

Summary: A single oral dose of 250 mg/kg produced 40% mortality while a dose of 500 mg/kg produced 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis. The EML oral dose is approximately 250 mg/kg.

Study title:RS43285 VBT: Oral EMLD study in mice

Key study findings: A single oral dose of 250 mg/kg caused severe clinical signs of subdued behavior, hunched stance, piloerection, hyperventilation and prostration in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing so the mice were euthanized. A single oral dose of 50 mg/kg produced no clinical signs.

Study no: AT3415, SS/050/85
Volume #, and page #: vol 16, p. 32
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: lot # 125SS0584, 100%
Formulation/vehicle:

Methods: The purpose of the study was to estimate the mean lethal oral dose. Survivors were euthanized after 14 days with no histological assessments made.CD1 mice (Charles River, Kent) were assigned to 2 treatment groups with 5 males and 5 females per group. After an overnight fast, the animals received a single oral dose of either 250 or 50 mg/kg.

Results: In the HD group, 1/5 m and 2/5 f showed marked clinical signs beginning approximately 5 minutes after dosing. Signs included "subdued behavior", prostration, hyperventilation, vasoconstriction, hunched appearance and piloerection. There was no improvement in the clinical status two hours after dosing, thus the animals were euthanized. In the surviving animals of this group, the signs remained pronounced until ~ 1 hour after dosing. Marked improvement was seen by 2 hours. No clinical signs were reported for the LD animals.

Summary: A single oral dose of 250 mg/kg caused severe clinical signs in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing so the mice were euthanized. A single oral dose of 50 mg/kg produced no clinical signs. The EML oral dose is approximately 250 mg/kg.

Study title: Intravenous EMLD study in mice

Key study findings: A single intravenous dose of 20 mg/kg produced no clinical signs. A single intravenous dose of 30 mg/kg produced clinical signs including hyperventilation, ataxia, piloerection, subdued behavior and prostration. Recovery time was within 1 hour of dosing. The LD50 is >30 mg/kg i.v.

Study no: AT3416, SS/029/85

Volume #, and page #: vol 16, 57
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April, 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: 125SS0584
Formulation/vehicle: water, sodium hydroxide, dextrose

Methods: Fasted CD1 mice (Charles River, Kent) were assigned to 2 groups of 5m and 5f per group. The mice were given a single intravenous dose of 20 mg/kg or 30 mg/kg.

Results: No signs were reported for the LD group. All mice receiving 30 mg/kg showed signs either immediately or within 10 minutes of dosing. Signs included subdued behavior, ataxia and piloerection. Subdued behavior was the most frequently reported sign. In 3/5m and 1/5f, subdued behavior was accompanied by hyperventilation, prostration and ataxia. Recovery time for all animals was reported to be within 1 hour of dosing.

Summary: A single intravenous dose of 20 mg/kg produced no clinical signs and 100% survival. A single intravenous dose of 30 mg/kg produced clinical signs including hyperventilation, ataxia, piloerection and subdued behavior. Recovery time was within 1 hour of dosing. The LD50 is >30 mg/kg i.v.

Study title: RS-43285: Intravenous EMLD study in rats

Key study findings: No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is > 30 mg/kg.

Study no: AT3417, SS/039/85
Volume #, and page #: vol 16, p. 83
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: 125SS0584, 100%
Formulation/vehicle: water, sodium hydroxide, dextrose

Methods : Fasted Sprague-Dawley rats (Charles River, Kent) were assigned to 1 group, with 5 males and 5 females. The rats received a single intravenous dose of 30 mg/kg.

Results: Clinical signs were observed in all animals immediately after dosing. The majority had slightly subdued behavior ± mild ataxia. Marked clinical signs were seen in 2/5m and 2/5f and included subdued behavior, ataxia, prostration, convulsions and hyperventilation. Recovery time was approximately 30 minutes from dosing, however, some animals showed subdued behavior and piloerection for the remainder of the day.

Summary: No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is > 30 mg/kg.

RS-43285-193/197/198: Comparative ELD study in rats

Key study findings: All animals showed signs of sedation, prostration, ataxia and dyspnea. The females receiving the racemic mixture had a later onset of signs (1.5 hours vs 12-38 minutes) compared to the animals receiving the enantiomers. The results are inconsistent with other studies that also found salivation, tremors and convulsions as well as earlier onset of signs with the racemic mixture.

Study no: AT6293
Volume #, and page #: Vol 27, p.244
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August 27, 1992
GLP compliance:
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: racemic mix(lot E6ML001), RS-43285-197 (S-, lot 19) and RS-43285-198 (R+, lot 18)
Formulation/vehicle: distilled water pH adjusted with sodium hydroxide.

Methods: Fifteen male and 15 female Crl:CD(SD)BR rats (Charles River, UK) were allocated to 3 treatment groups of 5 males and 5 females. The animals were given single oral doses of 250 mg/kg of either the racemic mixture or one of the enantiomers. The animals were observed for clinical signs and were weighed on days 1,8 and 15 of the study period. After euthanasia, gross observations were made. No histological examination was made.

Results: One female was euthanized 3 hours after receiving the racemic mixture. The dog showed severe clinical signs of prostration, subdued behavior, ataxia and dyspnea. Signs of subdued behavior and dyspnea began from 12-38 minutes after dosing in most animals except the females receiving the racemic mixture. That group of animals began showing signs approximately 1.5 hours after dosing. Ptosis and subdued behavior were present in most animals to approximately 3 hours. Signs had resolved by Day 2. Body weight data was not presented. Essentially the only data presented was that contained in the text of the report. It is not possible to assess the incidence, duration or severity of the signs from the data presented.

RS-43285 REJ: One month intravenous toxicity study in rats AT3280
Key study findings: Immediate salivation, sedation and convulsions followed iv administration of 25 mg/kg to both sexes of rats. Increased liver weight and decreased uterine weight were seen in drug-treated females. At 25 mg/kg 1/12 males and 1/12 females died. Increased spleen and adrenal weights were seen in the drug-treated males. No NOEL was determined for the organ weight effects in either sex.

Study no: AT3280

Volume #, and page #: volume 15, p.160
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August, 1984
GLP compliance: statement included (last page of appendices)
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: RS-43285-193, batch number 12, 100%
Formulation/vehicle: dextrose adjusted to pH 4. Also used as vehicle control.

Methods: Sprague-Dawley (Charles River, UK) rats, 12/sex/group were given daily intravenous doses of ranolazine of 0, 1,5 or 25 mg/kg for 28 days. The rats were observed daily for signs, twice a week for body weight and weekly for food consumption. Ophthalmoscopic exams were performed on all rats prior to study commencement and again during the fourth week of the study. Blood samples for hematology and clinical chemistry were collected from six animals per sex from all groupspre-study and from all animals during the 4th week of the study. Urinalysis was performed on samples obtained over a 4 hour period in metabolism cages from 6 animals/sex/group before the study and again during the 4th week of dosing. Full post-mortem exams were conducted on all rats. Certain organs were weighed and a standard list of tissues was collected for histopathological evaluation. Standard sections were stained with hematoxylin and eosin. Frozen sections of formaldehyde fixed liver were stained with Oil Red O for the presence of neutral lipid.

Results: Unscheduled mortality was seen in 1 HD male, one minute after dosing day 4 and 1 HD female immediately after dosing day 26. Signs for the male immediately before death were salivation and convulsions. Signs reported for the female included subdued behavior, salivation and convulsions. Signs were observed regularly for HD males from the first week to the end of the study and from week 2 in HD females to the end of the study. Convulsions, salivation and subdued behavior were seen regularly in a " small number" of animals immediately after dosing with recovery reported to be 5-15 minutes. Records of observation were not provided.

HD males gained on average 6% less than the control group. LD and HD females gained on average 10% and 4% less than the control group, respectively. There was no apparent difference in food consumption between the groups.

RBC count and HCT in MD and HD females were significantly decreased compared to the control and LD groups. This is summarized in the reviewer's table below.

Reviewer's summary of hematology changes in female rats

dose	RBC x 10^{12}/l	HCT ratio
Pre-dose all	6.72±0.31	0.409±0.020
0	7.24±0.20	0.421±0.017

2	7.22±0.28	0.421±0.016
5	6.85**±0.41	0.403*±0.021
25	6.85**±0.22	0.406*±0.010

There were no toxicologically significant clinical chemistry changes.
Absolute and normalized spleen and adrenal weights were increased in the drug-treated males.
Liver weight was increased and uterine weight decreased in treated females.

RS-43285 REJ : One Month Intravenous Toxicity Study in Rats

Group Mean Organ Weights (g)

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituit.	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Bodyweight
MALES														
1	0	Mean	2.02	1.24	4.63	0.013	11.74	0.73	2.69	0.78	0.067	0.62	0.022	333
		SD	0.08	0.13	0.76	0.002	2.11	0.16	0.38	0.11	0.013	0.08	0.003	34
2	1	Mean	2.00	1.25	4.54	0.013	11.46	0.74	2.80	0.81	0.070	0.61	0.023	333
		SD	0.09	0.10	0.64	0.002	1.29	0.26	0.20	0.14	0.015	0.15	0.004	34
3	5	Mean	2.03	1.29	4.71	0.015*	12.64	0.88	2.83	0.88	0.079	0.60	0.024	342
		SD	0.06	0.13	0.43	0.002	1.66	0.26	0.15	0.12	0.014	0.05	0.004	18
4	25	Mean	2.03	1.22	4.60	0.013	11.42	0.79	2.74	0.88	0.075	0.62	0.020	330
		SD	0.09	0.08	0.31	0.002	1.52	0.25	0.29	0.13	0.016	0.09	0.005	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituit.	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Bodyweight
FEMALES														
1	0	Mean	1.85	0.89	0.156	0.015	7.91	0.75	1.83	0.55	0.083	0.49	0.021	217
		SD	0.05	0.04	0.034	0.003	0.92	0.15	0.15	0.07	0.010	0.12	0.004	15
2	1	Mean	1.87	0.86	0.162	0.015	8.18	0.62*	1.88	0.60	0.077	0.52	0.021	215
		SD	0.12	0.08	0.021	0.002	1.06	0.21	0.20	0.08	0.012	0.12	0.003	16
3	5	Mean	1.88	0.87	0.162	0.015	8.77*	0.61*	1.90	0.56	0.075	0.49	0.021	216
		SD	0.07	0.08	0.025	0.002	0.76	0.18	0.17	0.07	0.012	0.08	0.005	16
4	25	Mean	1.84	0.86	0.158	0.014	8.69	0.64	1.88	0.61	0.078	0.52	0.019	217
		SD	0.04	0.08	0.020	0.002	1.14	0.14	0.14	0.11	0.008	0.05	0.005	12

< 0.05

43285 REJ : One Month Intravenous Toxicity Study in Rats

Group Mean Organ Weights as a Percentage of Bodyweight

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary x1000	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Bodyweight
MALES														
1	0	Mean	0.62	0.37	1.39	3.92	3.51	0.22	0.81	0.23	0.020	0.19	6.70	333
		SD	0.05	0.03	0.19	0.58	0.37	0.04	0.06	0.02	0.004	0.03	0.89	34
2	1	Mean	0.61	0.38	1.37	3.94	3.45	0.22	0.85	0.24	0.021	0.19	6.81	333
		SD	0.06	0.03	0.22	0.96	0.29	0.08	0.06	0.03	0.004	0.04	1.38	34
3	5	Mean	0.60	0.38	1.38	4.38	3.70	0.26	0.83	0.26	0.023	0.17	7.11	342
		SD	0.04	0.04	0.13	0.53	0.51	0.07	0.05	0.04	0.004	0.02	1.03	18
4	25	Mean	0.62	0.37	1.40	3.96	3.46	0.24	0.83	0.27*	0.023	0.19	6.18	330
		SD	0.03	0.02	0.15	0.67	0.36	0.07	0.07	0.04	0.004	0.02	1.73	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary x1000	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Bodyweight
FEMALES														
1	0	Mean	0.86	0.41	0.072	5.83	3.67	0.35	0.84	0.26	0.038	0.23	9.54	217
		SD	0.05	0.03	0.015	1.42	0.45	0.08	0.04	0.04	0.005	0.06	1.68	15
2	1	Mean	0.87	0.40	0.076	7.13	3.82	0.29	0.88	0.28	0.036	0.24	9.93	215
		SD	0.05	0.04	0.011	0.90	0.49	0.08	0.07	0.02	0.006	0.04	1.28	16
3	5	Mean	0.87	0.40	0.075	6.98	4.12*	0.28*	0.88	0.26	0.035	0.23	9.78	216
		SD	0.06	0.04	0.008	1.29	0.32	0.08	0.04	0.03	0.004	0.04	2.17	16
4	25	Mean	0.85	0.40	0.073	6.46	4.02*	0.30	0.87	0.28	0.036	0.24	8.73	217
		SD	0.04	0.03	0.008	0.95	0.53	0.07	0.05	0.05	0.003	0.03	2.37	12

The semi-quantitative urinalysis results were presented with acronyms that were undefined. It does not appear however, that there were discernible effects of drug treatment upon the results.

Study title: RS 43285 DCJ: Maximum tolerated intravenous dose study in dogs

Key study findings: Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received their dose and for approximately 15 minutes thereafter. At 20 mg/kg/day, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting increased tolerance to the dose, induction of metabolism or increased clearance. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle of the heart was found on gross necropsy. The only data from the ECGs was heart rate. The dose of 20 mg/kg/day was tolerated for 21 days by the 1 female who received it.

Study no: AT3844
Volume #, and page #: vol 17, p. 323

Conducting laboratory and location: Syntex research, Scotland
Date of study initiation: May 31, 1984
GLP compliance: preliminary, non-GLP
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot 111SS0284, >99%
Formulation/vehicle: 2% buffered solution of dextrose, sodium hydroxide and water.

Methods Two pairs of one male and one female Beagles were given once daily intravenous injections on the following schedule:

Pair 1- 7 days at 10 mg/kg/day
7 days at 20 mg/kg/day
Male- 1 day at 40 mg/kg/day
Female- 21 days further at 20 mg/kg/day
Pair 2 – 15 days at 20 mg/kg/day
Body weight was recorded twice weekly, food consumption daily. ECGs were performed at weekly intervals in pair 1 and before dosing and on days 9 and 15 in pair 2. At these times, ECG traces were taken before dosing, 1,6 and 24 hours after dosing. On day 9, the pair 2 traces were taken before dosing and 5 minutes after.

Blood samples were collected pre-study. Pair one was sampled at unspecified intervals while the second pair was sampled day 15 of dosing. Hematology and clinical chemistry parameters were analyzed. All dogs were examined at necropsy. No histopathological analysis was done.
Results: Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received their dose and for approximately 15 minutes thereafter. At 20 mg/kg/day, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting increased tolerance to the dose. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle of the heart was found on gross necropsy.
The only data presented from the ECGs was heart rate. Given the very small n of the study, the heart rate, hematology and clinical chemistry data is not particularly helpful.

Study title: RS 43285 DCT: Maximum tolerated oral (intubation) dose study in dogs

Key study findings: Signs reported were sedation, ataxia, muscle tremors, vomiting, salivation and prostration. Convulsions were reported for a male dog who was then euthanized in extremis. Signs lasted for up to 6-7 hours with higher dosages. The dose of 150 mg/kg following the periods of lower dosages was the estimated lethal dose. The 80 mg/kg/day dose is the estimated maximum tolerated dose for an oral dosing study.

Study no: AT 3845
Volume #, and page #: vol 17, p. 359
Conducting laboratory and location: Syntex Research, Scotland

Date of study initiation: May 15, 1984
GLP compliance: preliminary, non-GLP report
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: sodium hydroxide and distilled water.

Methods Two pairs of Beagles, one male and one female per pair, were intubated orally once each day. Pair one received 50 mg/kg/day for one week followed by 100 mg/kg/day for a week. Both dogs then received an additional single oral dose of 150 mg/kg/day. Pair two was dosed at 100 mg/kg/day for 9 days. This was discontinued due to marked clinical signs. After a seven week recovery period treatment was resumed at 80 mg/kg/day for 2 weeks. Pair 2 was weighed twice weekly during the 2 weeks of 80 mg/kg/day treatment. Food consumption was estimated daily. ECGs were performed before dosing and 1, 6 and 24 hours after dosing in pair 2 on days 1 and 14. Blood samples for hematology and clinical chemistry were collected from the pair 2 dogs pre-test and week 2. After euthanasia, gross necropsy was carried out. No histopathological evaluation was performed.

Results: Single doses of 50 mg/kg and 80 mg/kg caused vomiting within 45 minutes of dosing. A single dose of 100 mg/kg in naïve animals produced no signs. Continued dosing at this level produced combinations of sedation, ataxia, muscle tremors, vomiting, salivation and on one occasion in the female, prostration. On day 9, both dogs were found prostrate and trembling 2 hours after dosing and the male appeared unaware of its surroundings. Partial recovery was seen within 2-4 hours.
A dose of 100 mg/kg following a week at 50 mg/kg caused sedation, muscle tremors, mild ataxia and staining of the mouth in the male. No signs were reported for the female. The male continued to show these signs for the rest of the week at this dose. The female vomited on most occasions within 60 minutes of dosing and showed sedation on several occasions 1-2 hours after dosing. Recovery was within 6-7 hours.
A single oral dose of 150 mg/kg after dosing at 50 and 100 mg/kg caused severe clinical signs in the male within 20 minutes of dosing. Signs included prostration, convulsions, irregular breathing and slightly decreased heart rate. The female showed sedation and muscle tremors with recovery within 4 hours. The male was euthanized in extremis.
Repeated daily doses of 80 and 100 mg/kg/day caused sedation, muscle tremors, mild ataxia, salivation, vomiting and prostration within 1 hour of dosing. Recovery took 3-5 hours.

RS-43285 DEJ: One month intravenous toxicity study in dogs.

Key study findings: This study was suboptimal in reporting and had few apparent findings of significance. The HD (20 mg/kg/day) animals showed signs of sedation, trembling, vomiting and hindlimb ataxia. ECGs were obtained but only raw heart rate data was presented.

Study no: AT3281
Volume #, and page #: Vol 15, p. 279
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: July 1984

GLP compliance: statement included (last page of appendices)
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lot number 125ss0584,
Formulation/vehicle: dextrose at pH = 4.

Methods: Three male and three female beagles (Crowley, Malvern, UK) per group were given daily intravenous injections of 0, 1,5 or 20 mg/kg/day of ranolazine each day for 28 days. Animals were observed daily for signs, weighed weekly and food consumption estimated daily. All animals had ophthalmic exams pre-study and during week 4 of the study. ECGs were obtained from each animal pre-study, day 1 and during week 4. During the study, ECGs were obtained before dosing, 5 minutes, 1 and 6 hours after dosing. Blood for clinical pathology was collected from each animal pre-study and during the 4th week of the study. Urinalysis was performed at the same time points using metabolism cages. All animals were given a post-mortem examination. Those surviving to scheduled euthanasia were terminated 24 hours after the final dose. As indicated in the histopathology inventory, various organs were weighed and a reasonably standard list of tissues was collected for histopathological examination.

Results: There was no unscheduled mortality. No clinical signs were reported for the control groups. Vomiting before dosing was reported for 1 LD male and 1 LD female day 7. The MD group had reported signs of subdued behavior after dosing on day 1 for 1 male and overnight vomiting day 2 for another male. Signs were reported predominantly for the HD animals. "Subdued behavior" was reported almost daily for all the HD animals. This began immediately after dosing and lasted from 5 minutes to 1 hour. There were also "frequent" reports of vomiting, trembling and hind limb ataxia. One female showed ataxia almost continuously during weeks 3 and 4. Conjunctival congestion was also noted after dosing on day 12 for a HD male and a HD female. No incidence tables were presented for the clinical signs.
Average body weight gain was dose-dependently decreased in the treated males. There was no apparent connection between treatment and body weight for the females. This is summarized in the reviewer's table.

Summary of weight changes

Dose group (mg/kg/day)	Avg male weight gain (kg)	Avg female weight gain (kg)
0	0.43	0.7
1	0.43	-0.1
5	0.17	0.7
20	0	0.4

Food consumption was decreased sporadically in the controls, LD and MD females, significantly so in the last week of the study for the LD and MD females.

ECGs: Only heart rate data was presented. The single animal data was averaged by the reviewer and is presented in the following table.

Reviewer's summary of reported ECG data: Average heart rate (beats per minute) at several times post-dosing

Sex and	Day 1	Week 4

dose (mg/kg)	bd	5 mins	1 hr	6 hr	bd	5 mins	1 hr	6 hrs
Males								
0	112	115	122	124	128	115	128	133
1	131	104	123	121	108	98	110	128
5	146	124	114	153	140	118	119	141
20	133	126	112	140	131	124	111	154
Females								
0	133	129	141	147	126	118	128	140
1	129	111	124	138	116	94	109	130
5	108	123	124	142	129	112	113	143
20	116	149	129	136	113	126	107	133

There do not appear to findings of toxicological significance in the hematology, clinical chemistry or urinalysis data. The urinalysis data was presented with acronyms for which there were no definitions.

Organ weights were presented as single animal data. The reviewer calculated averages for organ weights provided as a percentage of body weight. No significant differences were apparent. However, percentage of body weight is very insensitive for small organs such as the pituitary and adrenal. There were 2 outliers in the control and LD group with regard to uterine weight. Because of this it cannot be determined if there was in fact a dose-related decrease in that organ's weight.

An ophthalmologist's report was not located.
A scant summary of histologic findings showed non-remarkable findings with the exception of meningo-encephalitis in one HD female. The sponsor suggests that this was of viral origin. This raises the question of the standards of care for a viral meningo-encephalitis to have found entry into the colony.

Study title: Oral investigative tolerance study in Beagle dogs with ranolazine administered three times daily.

Key study findings: This study provides limited data. At 60 mg/kg signs after dosing were sedation, salivation, vomiting, ataxia and trembling. Blood vessel dilation (pink ears) was also reported. It is not clear whether hypotension was the sole cause of the subdued behavior, recumbent animals, thrashing and barking.

Study no: AT6436 SS/14/92
Volume #, and page #: vol 28, p. 4
Conducting laboratory and location: Syntex Research, Edinburg, Scotland
Date of study initiation: June 12, 1991
GLP compliance: statement not located
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lot 14 E6-ML-001 and E7-ML-001

Formulation/vehicle: possibly given in gelatin capsules

Methods Two male and 2 female dogs were given escalating doses of ranolazine, three times a day on the following schedule:

Days 1-7	25 mg/kg/tid ranolazine
Days 8-14	40 mg/kg/tid ranolazine
Days 15-21	50 mg/kg/tid ranolazine
Days 22-35	60 mg/kg/tid ranolazine

Animals were observed daily for signs, weighed weekly and food consumption was monitored each day. One male and 1 female had ECGs done pre-trial and again on days 1,7,8,14,15,21,22,28,29 and 35 1 and 6 hours after the morning dose.
The same two animals had blood samples collected pre-test, days 7,14, 21,28 and 35. Standard hematology and clinical chemistry were monitored. Animals surviving to the end of the treatment period were euthanized the day after the last dose. No organs were weighed. Histopathological examination was confined to the stomach of one male who was euthanized ahead of schedule. Blood samples for determination of ranolazine in plasma were taken from 1 male and 1 female on days 1,7,14,21 and 28. Day 35 samples were taken from the surviving animals. Timepoints taken were predose, 20 minutes, 40 minutes, 1,2,4,6 and 8 hours after the morning dose. Samples were analyzed by HPLC with fluorometric detection.

Results: One male was euthanized day 29, 24 hours after his last dose due to marked clinical signs of subdued behavior, thrashing legs, trembling and tachypnea lasting ~1.25 hours. The animal remained subdued for another 6 hours. Plasma drug levels were determined but not reported. Clinical signs reported at 25 and 40 mg/kg/tid included green feces and occasional vomiting. At 50 mg/kg/tid, animals occasionally salivated before and/or after dosing. One animal trembled approximately 1 hour after the morning dose. At 60 mg/kg/tid, salivation and peripheral vasodilation (pink ears) became pronounced. Trembling and subdued behavior were also noted. One female was observed on day 35, ~ 1 hour after dosing, lying on its side, legs flaying, barking, trembling and salivating. The sponsor also reported the animal as very subdued. The report is somewhat unclear as it states:

> …This lasted approximately 4 minutes and the animal appeared to be recovered 3 hours later. A blood sample and ECG were taken for diagnostic purposes only at this time but the results have not been reported here as they did not appear to be drug related.

It is difficult to say if there is a body weight effect as there was no untreated or vehicle group for comparison and a small sample size. There was no apparent change in body weights over the duration of the study.
Only heart rate data was presented from the ECGs. As there was 1 animal per sex for a total of 2 animals, with no untreated control for comparison, no conclusions can be drawn from the data.
The hematology and clinical chemistry data were for 1dog/sex. Interpretation is difficult. Given the small sample size, the plasma drug data should not be overinterpreted. It can be said that $AUC_{0\text{-}8}$ (ng.hr/ml) increased with increasing dose.
No gross lesions were reported. The one stomach that was examined histopathologically had multiple small irregular erosions at the gastroesophageal junction that were considered to be unrelated to treatment.

Summary: This underpowered study cannot be given much weight. However, certain observations are interesting: blood vessel dilation (pink ears). It is not clear whether hypotension could be the sole cause of the subdued behavior, recumbent animals, thrashing and barking.

Study title: Four week investigative study in dogs

Key study findings: This single dose study had few findings of toxicological significance.

Study no: AT6543; SS/029/93
Volume #, and page #: vol 28, p.57
Conducting laboratory and location: Syntex Research, Edinburgh, Scotland
Date of study initiation: August 17, 1993
GLP compliance:
QA report: yes () no ()
Drug, lot #, radiolabel, and % purity: batch number E3-NE-002
Formulation/vehicle: tablets

Methods: The study was originally to evaluate local gastrointestinal effects of a sustained release tablet formulation of ranolazine free base. Two male Beagles were assigned to the control group and 4 to the treatment group. The animals were dosed once a day, approximating the target dose of 68.2 mg/kg/day. The sponsor states that this is equivalent to 80 mg/kg/day of the dihydrochloride that was used in a previous 3 month oral study in dogs. Animals were observed for signs, weighed weekly and food consumption measured daily. ECGs were performed pre-trial and on days 3 and 24 of dosing at 3,6 and 24 hours after dosing. Blood samples for plasma ranolazine determination were taken 1,7 and 28 days of dosing at 1,3,5,8,12 and 24 hours after dosing. Samples for clinical pathology were taken before dosing and on day 22 of the study. Animals were euthanized ~3 hours after the last dose, gross observations made, organ weights determined, and samples collected.

Results: There were no apparent differences in weight gain although the treated dogs did eat less than the control animals. Only heart rate data was shown from the ECGs. The rate for the control animals went down in the first 24 hours then increased at two points of determination in week 4. The treated animals showed decreased heart rates day 3. In week 4, 3 continued to show decreased rates while one showed an increase. Organ weight data was provided. However, the small sample size and degree of variability render the data of little value. Plasma level determination of drug exposure showed an increase in AUC $_{0\text{-}24}$ (ng.hr/ml) from day 1 to day 7: 19,500±9450 vs 25,100±13200 respectively. There was a slight decrease evident day 28: 20,500±17300 ng.hr/ml. No gross findings were reported. The only histopathology results provided was the statement "Histopathological examination of the stomach and intestines revealed no pathology."

Summary: It is difficult to say from the data presented whether the decreased heart rates seen in the treated dogs were within the realm of normal variability or treatment-related. This single dose study had few findings of toxicological significance.

Study title: RS-43285 DHT: three month oral toxicity study in dogs

Key study findings: This study was incompletely reported. Although ophthalmic exams were conducted there was no statement from an ophthalmologist. Although ECG tracings were reportedly done, only single animal heart rate data was presented. In both sexes of drug treated animals heart rate was decreased at the 1 hour post-dose observation time in week 1. Clinical signs were noted for doses ≥25 mg/kg, and included salivation, vomiting, ptosis, glazed eyes, conjunctival congestion, sedation, ataxia, trembling and convulsions. "Subdued behavior" was especially apparent in the first month. Inconsistent with the 6 month dog study, the signs reported in this study began from 10-30 minutes after dosing. Significant changes in hematology, clinical chemistry and urinalysis are not apparent. Absolute and normalized testicular and adrenal weight for the treated male dogs was increased over control but not in a dose-dependent fashion. Uterine weight of the drug-treated females was decreased compared to the control group. The presentation of pathology findings was confusing and raised questions as to the consistency of observations made. The manufacturing and stability data in Appendix L noted that placebo analysis indicated an RS-43285-193 content of less than 6.1 x 10^{-5} to less than 1.1 x 10^{-4} % w/w. It is not made clear if this means that ranolazine was not present or was present in levels near the limits of detection/quantitation.

Study no:AT3440, SS/028/85
Volume #, and page #: vol 16, p 109
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 11, 1984
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: 125SS0584
Formulation/vehicle: aqueous

Methods: 28 dogs from Ciba Geigy Pharmaceuticals (Cheshire) and 2 dogs from E.G. Crowley (UK) were assigned to 5 treatment groups of 3 males and 3 females per group. The dogs were dosed once a day for 91-93 days with 0, 5, 25, 60 or 80 mg/kg/day of drug. Animals were monitored daily for signs and food consumption, weekly for body weight, ophthalmoscopy (pre-treatment and week 13), and electrocardiography (pre-treatment, day 1, week 6 and week 13). Tracings were made pre-dose, 1, 6 and 24 hours after dosing. Blood was collected for clinical chemistry and hematology before dosing, and after 4, 8 and 12 weeks of dosing. Urinalysis was performed at the same time points via use of metabolism cages.
Those surviving to the end of the dosing period were euthanized 24 hours after the last dose. Specified organs were collected and weighed.
Results: No unscheduled mortality was reported. Signs were noted for the 3 highest dose groups with the two highest dose groups, 60 and 80 mg/kg, most frequently involved. Subdued behavior was noted frequently, especially in the first month of dosing. Other signs included vomiting, salivation, trembling, conjunctival congestion, ptosis, glazed eyes and ataxia. A combination of these signs was elicited week 13 during the blood sampling, ECG and ophthalmoscopy procedures. Convulsions were limited to the HD group. Other details reported for the HD group included pupils dilated (33%), pupils non-responsive to light (72%) and unusual skin tone of pink or blue (34%). Signs in the 25 mg/kg group were vomiting, ptosis, glazed eyes, ataxia and

subdued behavior. No signs were reported for the 5 mg/kg group. Signs were reported to begin from 10-30 minutes after dosing.

The mean weight change per group was a decrease in body weight. The only exceptions were the 5 mg/kg and 80 mg/kg females who showed slight mean gains.

Reviewer's Summary of Weight Changes (Kg) Calculated from Data Presented

	Dose group (mg/kg/day)				
	0	5	25	60	80
males	-0.44	-0.40	-0.99	-0.89	-0.82
females	-0.73	+0.17	-0.19	-0.05	+0.23

The hematology, clinical chemistry and urinalysis data were presented as single animal data. There were no apparent effects in any of these.

There was no statement from an ophthalmologist as to the ophthalmic findings and no indication that an ophthalmologist had conducted the evaluations.

ECGs- Only single animal HR data was presented. A decrease in heart rate was seen 1 hour after dosing in all drug-treated groups. This effect was apparent at Day 1 in both sexes and week 6 also in females. This is summarized in the reviewer's tables below.

Reviewer's Summary of Heart Rate Changes for Males

Day 1					
Dose mg/kg	Pre-trial	predose	1hr	6hrs	24hrs
0	118	130	149	115	130
5	106	124	109	104	102
25	95	106	85	108	121
60	116	105	99	101	102
80	157	147	124	122	138

Week 6					
0		130	122	122	131
5		108	96	106	105
25		95	89	102	95
60		108	101	121	110
80		130	126	135	141
Week 13					
0		130	136	124	112
5		100	99	103	109
25		83	81	107	85
60		96	107	94	101
80		117	131	124	118

Reviewer's Summary of Heart Rate Changes for Females

Day 1					
Dose mg/kg	Pre-trial	predose	1hr	6hrs	24hrs
0	127	126	121	138	127
5	125	120	109	106	115
25	133	140	121	136	134
60	125	129	102	129	113
80	130	110	105	106	102
Week 6					
0		105	108	104	107
5		117	97	108	91
25		128	108	144	114
60		136	128	128	149
80		139	111	121	131
Week 13					
0		128	114	124	107
5		92	94	99	90
25		110	120	121	126
60		126	123	114	114
80		111	96	112	125

The histopathology was presented as selected findings for single animals. The protocol states that histopathological evaluation will be performed on all animals of both sexes from all groups (p.211). Clearly this was not done as several of the animals are listed with the designation that no histopathology was done. An amendment to the protocol says that as no compound-related lesions were found in the HD animals, it was decided not to evaluate the other groups. For the control males it was specifically noted that in the testes there was spermatogenesis present and no pathology noted. This designation was not made for every male examined nor are there comments about the testes for every male. The inconsistency is confusing. Does that mean the testes were not examined or were abnormalities not reported? One designation for one HD male notes that there was a localized area of tubular germinal epithelial degeneration which was then immediately qualified as a possible post-mortem artifact.

It was noted that pleural lesions seen at necropsy frequently showed changes consistent with Filaroides hirthi infestation (vol 16, p.128). The presence of this parasite may well confound interpretation of clinical chemistry and hematology results.

Organ weight data was presented for individual animals. In an underpowered study such as this one would not expect to be able to discern differences between groups. The reviewer calculated means for absolute and normalized organ weight from the data presented. This is summarized in the table below. The absolute and normalized weight of testes in the drug-treated groups was more than that of the control group. No pattern was discernible. Absolute and normalized adrenal weight was also increased

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (testes)	13.12	14.06	15.2	13.06	15.66
Normalized (testes)	0.95	1.13	1.20	1.02	1.088
Absolute (adrenal)	0.74	0.72	0.835	0.85	0.87
Normalized (adrenal)	0.054	0.058	0.066	0.067	0.060

Uterine weight was also decreased in all drug-treated groups. Adrenal weights in the females were not discernibly affected.

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (uterine)	8.05	6.86	3.41	4.78	2.89* 4.03**
Normalized (uterine)	0.75	0.59	0.29	0.40	0.24* 0.34**

* the sponsor had a footnote for one individual measurement "left horn only". ** the asterisked animal was omitted from the calculation

Study title:RS-43285 DJC: Six month oral toxicity study in dogs

Key study findings: Signs were primarily reported for doses ≥25 mg/kg but appeared in all groups with no NOEL identified. Signs included mydriasis with loss of PLR, glazed eyes, ptosis and other signs of sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups and was no longer reported for the LD group. Rouleaux was reported for the MD and HD males and 2 HD females. Adrenal weights were slightly increased in the HD males while testicular weight was decreased in the HD group. Pituitary weight was increased in females in a dose-related manner. Although

ECG tracings were obtained only single animal heart rate data was presented with no reporting of ECG intervals.

Study no: AT4050, SS/002/88
Volume #, and page #: vol 19, p 5.
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: October 1986
GLP compliance: statement included in the appendix
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: "various mixtures" containing lactose and magnesium stearate provided in gelatin capsules. Vehicle capsules contained lactose and magnesium stearate

Methods: Sixteen male and 16 female dogs (Alpha Sirius, Malvern, U.K.) from 12-16 months of age, with the exception of male #405 who was 24 months of age, were used. The dogs were assigned to 4 treatment groups with 4 males and 4 females per group. The dogs were dosed orally once a day for 26 weeks. Doses used were 0, 5, 25 and 60 mg/kg/day. ECGs were recorded pretest, day 1, month 3 and month 6. The tracings were taken pre-dose, 1, 6 and 24 hours after dosing. Dogs were sampled for hematology, clinical chemistry and urinalysis pre-dose, 4, 13 and 25 weeks after dosing started. The eyes were examined pre-test and again at 3 and 6 months. Plasma profiles were taken from all dogs to determine evidence of absorption. Samples for this were collected at 0.5, 1,2,4 and 8 hours after dosing. The results were reported separately. At necropsy, various organs were weighed and the standard tissues collected for histopathology.

Results: Signs were mostly at ≥ 25 mg/kg and included mydriasis with decreased or loss of pupillary light reflexes, glazed eyes, ptosis and sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups. The mydriasis appeared within several hours of dosing and persisted throughout the working day.
There were no discernible patterns in the body weight changes for either sex**.**
The only ECG data that was presented was heart rates for individual animals. The reviewer averaged the individual heart rate data provided. The summary is shown below.

Reviewer's Summary of mean heart rate data for the 6 month dog study: males

Dose (mg/kg/ day)	Pre-study	Day 1 (hrs post-dose)				3 months (hrs post dose)				6 months (hrs post-dose)			
[redacted]		BD	1	6	24	BD	1	6	24	BD	1	6	24
0	174	155	137	148	134	125	137	140	132	126	126	137	115
5	154	148	136	137	115	132	112	139	133	143	143	157	151
25	127	123	110	122	121	114	102	117	103	114	103	112	109
60	150	123	107	120	122	103	108	113	110	107	112	114	104

Reviewer's Summary of mean heart rate data for the 6 month dog study: females

Dose (mg/kg/ day)	Pre-study	Day 1 (hrs post-dose)				3 months (hrs post dose)				6 months (hrs post-dose)			
[redacted]		BD	1	6	24	BD	1	6	24	BD	1	6	24
0	133	130	112	125	113	104	112	109	116	117	117	124	110
5	135	128	117	141	138	143	116	128	114	115	124	139	125
25	142	120	113	124	116	114	104	118	117	108	101	125	125
60	149	130	135	132	156	118	122	115	131	105	109	118	112

In the hematology section, rouleaux were noted for 2 MDm dogs, all HD males and 2 MDf. Reticulocytosis appeared sporadically in all groups. One HD female sporadically showed the highest degree of reticulocytosis for the study.

Weight of the adrenal glands and testes were affected in HD males. Pituitary gland was affected in females. Relative organ weights were presented, but without any indication what the comparison was. Organ weight changes are summarized in the reviewer's table below.

Reviewer's summary of organ weight changes

Males				
	Dose group (mg/kg)			
	0	5	25	60
Adrenals absolute weight	0.70±0.07 (left) 0.77±0.09 (rt)	0.70±0.05 (left) 0.80±0.20 (rt)	0.74±0.12(left) 0.76±0.11(rt)	0.81±0.09(left) 0.88±0.12(left)
Relative weight	0.006±0.001(left) 0.007±0.001(rt)	0.006±0.000 0.006±0.001	0.007±0.001 0.007±0.000	0.007±0.001 0.008±0.002
Testes Absolute weight	13.43±.8 (left) 13.52±.52 (rt)	14.31±2.58 (left) 13.18±2.12 (rt)	13.26±1.14 (left) 13.15±1.02 (rt)	10.38±0.68 (left) 10.03±0.76(rt)
Relative weight	0.116±0.007(left) 0.117±0.007(rt)	0.115±0.011 0.106±0.010	0.116±0.011 0.115±0.007	0.090±0.009 0.087±0.009
Females				
Pituitary Absolute weight Relative	0.051±0.015 0.004±0.0001	0.073±0.014 0.0006±0.0000	0.071±0.029 0.0006±0.0003	0.080±0.011 0.0007±0.0002

Summary: Rouleaux formation is a grouping of erythrocytes that resembles a stack of coins. Degree of rouleaux tends to parallel the erythrocyte sedimentation rate, an indicator of inflammation or may be associated with a qualitative change in serum globulins (Duncan and Prasse). Rouleaux may appear to a mild degree in a healthy dog and to a more marked degree in inflammatory or neoplastic processes. The decrease in testicular weight in the HD males is of interest in light of the findings in the fertility study in which male rats showed decreased fertility.

Study title: One year oral toxicity study in dogs

Key study findings: The reporting is suboptimal. The signs in this study, seen primarily at 60 mg/kg/day, were significant in that salivation, sedation, trembling, convulsions, behavioral changes, ataxia and skin conditions were noted. The sponsor ascribes these to hypotension and/or cardiovascular collapse but does not present data to support this. Minimal information was presented concerning a possibly drug-related skin condition in the HD group. Ophthalmic examinations were conducted by a "veterinary consultant" and it is not made clear if this was a veterinary ophthalmologist.

Study no: AT6971/SS/028/94
Volume #, and page #: vol 30, p.3
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: March 23, 1993
GLP compliance:
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: capsules

Methods: Beagles (5/sex/group) were dosed once a day for 1 year with 0,10,25 or 60 mg/kg/day of ranolazine. Dogs were observed each day for signs and food consumption. Body weight was recorded weekly. Ophthalmic exams were performed pre-test and again during months 6 and 12 of the study by an "external veterinary consultant". Electrocardiography was conducted on all animals pre-test, day 1 and during the 5^{th} and 11^{th} month of dosing. ECGs were recorded before dosing and 1,6 and 24 hours after dosing. Blood was collected from all animals for clinical pathology pre-test and again after 1,3,6 and 12 months of dosing. Urinalysis was performed at similar timepoints. Plasma samples were collected after 6 and 12 months of dosing at 0, 0.5, 1,2,5 and 8 hours post dose. At necropsy, gross observations were made, some organ weights taken and tissues collected.

Results: Three out of 5 HD m developed an unspecified skin condition, which after extensive and inconclusive diagnostic work-up was reported to resolve spontaneously. A dermatologist evaluated the cases and came to the conclusion that

APPENDIX E
Ranolazine : One Year Oral Toxicity Study in Dogs
Dermalotogists Report

Mr W D Taylor

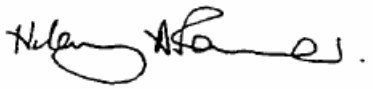
2.

Routine chemistries, haematology and urinalysis appear unremarkable.

Having ruled out the common causes of a non-pruritic alopecia (i.e. demodicosis, dermatophytosis) diagnosis rests on the histopathological findings. These suggest an inflammatory disease. With the focal epidermal necrosis it would be impossible to rule out the possibility of a drug related toxicity, an immune mediated reaction or external contact or irritant dermatitis. A combination of events or factors may be involved, and to investigate this further a programme of elimination/provocation would be required.

I would be interested to see any further cases of this dermatosis as and when they occur. It is possible that by biopsying an "earlier" lesion we would get a more representative picture of the disease process.

Yours sincerely



Hilary A Farmer BVM&S, CertSAD, MRCVS
Resident in Dermatology

Signs were reported only for the HD group. Salivation was seen most frequently and in the females subdued behavior was also seen frequently (starting 1 hour after dosing and lasting ~ 1 hour). Additional signs were reportedly sporadic in occurrence and included subdued behavior, nervous or wary behavior, ataxia, lying or sitting in the corner of the pen, trembling and watery, glazed or half-closed eyes. Onset was reported as 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Three out of 5 females and 3/5 males in the HD group had convulsions on several occasions, between 21 and 94 minutes after dosing. The convulsions lasted 2-5 minutes during which time the animals were in lateral recumbency, paddling, unaware of the surroundings and panting. One out of 5 LD males convulsed on three occasions, twice before dosing and once 6.5 hours after dosing, in each case in association with some procedure.

There were no differences in body weight gain in the data as reported.

Study Month	Dose mg/kg/day		Na mmol/l	K mmol/l
Pretrial	All Groups	mean	150	4.19
		SD	2	0.24
1 Month	0	mean	145	3.99
		SD	1	0.18
	10	mean	144	3.92
		SD	2	0.17
	25	mean	145	4.13
		SD	1	0.31
	60	mean	145	4.13
		SD	1	0.21
3 Month	0	mean	148	3.92
		SD	1	0.11
	10	mean	147	4.05
		SD	1	0.14
	25	mean	148	3.99
		SD	1	0.14
	60	mean	149	4.09
		SD	1	0.16

Ophthalmic findings were reported as "…three animals, one of which was a control had any findings (corneal opacity, retinal folds and cataract). None of the findings was considered to be related to the treatment…"

Study Month	Dose mg/kg/day		Na mmol/l	K mmol/l
6 Month	0	mean	145	3.72
		SD	1	0.27
	10	mean	144	3.89
		SD	1	0.13
	25	mean	144	3.77
		SD	1	0.20
	60	mean	**148	3.88
		SD	2	0.27
12 Month	0	mean	148	3.7
		SD	0	0.3
	10	mean	147	4.2
		SD	2	0.3
	25	mean	148	4.0
		SD	1	0.2
	60	mean	150	*4.2
		SD	1	0.4

Electrocardiography: only the heart rates were reported. Dose dependent effects were not apparent in the data presented.

Clinical chemistry: there were no changes of biological significance apparent in the hematology data. Serum sodium was slightly increased in the HD males at 3, 6 and 12 months. Serum sodium and potassium were increased in the HD females at 3,6 and 12 months.

Absolute and normalized adrenal weights from all drug-treated groups of males and from the HD females weighed on average more than the control organs. Absolute and normalized kidneys in drug-treated animals of both sexes weighed more than those of the controls. The absolute and normalized weights of the uterus in the MD and HD groups were less than the control weights.

Table 7

Ranolazine: One year Oral Toxicity Study in Dogs

Organ Weights Adjusted for Bodyweight Group Summary - Males

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Pituitary	Prostate	Spleen	Testes Left	Testes Right	Thymus	Thyroid Left	Thyroid Right
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14732	0.696	0.761	81.118	138.348	31.374	31.680	439.222	0.0679	11.691	75.555	13.195	12.831	19.495	0.384	0.381
	se	0.388	0.053	0.061	2.39	12.824	1.85	1.705	21.914	0.0073	2.041	10.203	0.837	0.687	2.166	0.048	0.036
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14450	0.903	0.94	83.939	163.268	37.323	36.098	440.522	0.071	17.388	104.884	11.132	12.183	20.250	0.355	0.345
	se	0.388	0.054	0.062	2.422	12.996	1.874	1.728	22.207	0.0074	2.069	10.339	0.849	0.696	2.195	0.049	0.037
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14858	0.765	0.821	81.016	135.285	32.948	32.875	428.809	0.0594	12.895	102.084	11.854	11.556	18.572	0.374	0.387
	se	0.388	0.053	0.061	2.401	12.884	1.858	1.713	22.015	0.0074	2.051	10.250	0.841	0.690	2.176	0.048	0.036
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14784	0.819	0.84	83.241	138.920	33.783	33.659	466.312	0.0657	12.786	81.119	13.716	13.663	19.540	0.445	0.457
	se	0.388	0.053	0.061	2.393	12.839	1.852	1.707	21.938	0.073	2.044	10.214	0.838	0.688	2.169	0.048	0.036

n = number of animals

LS mean = least square mean

se = standard error of least square mean

Significance level of comparison with control (0 mg/kg/day) using William's test * = p<0.05

Table 6
Ranolazine: One year Oral Toxicity Study in Dogs/
Organ Weights (g) Group Summary - Females

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Ovary Left	Ovary Right	Pituitary	Spleen	Thymus	Thyroid Left	Thyroid Right	Uterus
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14160	0.71	0.75	74.92	105.31	27.01	26.9	431.15	0.66	1.00	0.088	74.17	16.79	0.34	0.4	13.73
	SD	1253.2	0.08	0.06	6.05	9.64	2.58	3.27	51.77	0.19	0.13	0.015	17.14	5.2	0.06	0.06	8.03
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	13314	0.76	0.79	81.86	106.55	28.02	28.25	386.83	1.08	0.9	0.08	87.41	14.51	0.33	0.45	14.34
	SD	1296.7	0.09	0.06	8.4	11	3.74	3.58	46.05	0.58	0.31	0.007	35.5	2.76	0.06	0.08	8.2
25	n	5	5	5	5	5	5	5	5	5	5	5	5	[illegible]	[illegible]	[illegible]	5
	Mean	12906	0.66	0.73	82	105.98	27.11	26.96	366.56	0.63	0.77	0.078	67.15				8.39
	SD	2265.2	0.03	0.1	3.98	7.61	3.13	2.79	43.43	0.21	0.16	0.004	24.24				3.99
60	n	[illegible]	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5	5	[illegible]	5	5	[illegible]	5	5	5
	Mean	14208	0.82	0.91	82.54	124.2	29.26	28.84	484.83	0.8	0.88	0.088	75.43	14.93	0.42	0.44	9.96
	SD	1618.4	0.14	0.14	6.82	14.84	1.57	1.71	81.97	0.25	0.54	0.016	15.46	4.82	0.12	0.07	6.94

Table 6
Ranolazine: One year Oral Toxicity Study in Dogs
Organ Weights (g) Group Summary - Males

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Pituitary	Prostate	Spleen	Testes Left	Testes Right	Thymus	Thyroid Left	Thyroid Right
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14732	0.7	0.76	81.15	138.59	31.41	31.72	440.07	0.068	11.66	75.43	13.17	12.82	19.62	0.38	0.38
	SD	381.2	0.05	0.13	4.86	19.8	3.2	3.88	61.77	0.023	3	27.44	2.69	2.55	6.84	0.1	0.1
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14450	0.89	0.93	83.6	160.86	36.93	35.69	432.17	0.07	17.74	106.08	11.36	12.29	18.98	0.35	0.35
	SD	1066.2	0.17	0.15	4.38	41.36	6.16	4.82	52.78	0.012	6.24	24.70	2.37	1.07	4.53	0.14	0.06
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14858	0.77	0.83	81.22	136.71	33.18	33.12	433.77	0.06	12.69	101.38	11.72	11.49	19.33	0.38	0.38
	SD	533.1	0.11	0.06	6.56	33.3	4.15	4.27	39.33	0.012	4.44	17.13	0.99	0.56	4.92	0.08	0.06
60	n	[illegible]	5	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5	5	[illegible]	5	5	[illegible]	[illegible]	5
	Mean	14784	0.82	0.84	83.34	139.65	33.9	33.78	468.86	0.066	12.68	80.76	13.65	13.63	19.93	0.45	0.45
	SD	201.9	0.15	0.19	5.15	11.71	2.42	2.38	63.68	0.015	4.01	18.98	1.31	1.18	8.43	0.08	0.1

Table 7
Ranolazine: One year Oral Toxicity Study in Dogs
Organ Weights Adjusted for Bodyweight Group Summary - Females

Dose mg/kg g/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Ovary Left	Ovary Right	Pituitary	Spleen	Thymus	Thyroid Left	Thyroid Right	Uterus
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14160	0.716	0.755	73.856	103.871	26.499	26.541	425.522	0.658	1	0.088	71.3	15.954	0.336	0.389	14.168
	se	0.742	0.044	0.044	2.591	4.717	1.101	1.258	25.762	0.161	0.155	0.0055	10.574	1.792	0.035	0.026	3.211
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	13314	0.759	0.792	*82.555	107.481	28.354	28.485	390.486	1.081	0.899	0.08	89.273	15.049	0.332	0.453	14.058
	se	0.742	0.044	0.043	2.569	4.677	1.092	1.248	25.545	0.16	0.153	0.0055	10.485	1.777	0.035	0.026	3.184
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	12906	0.658	0.726	*83.537	108.064	27.843	27.481	374.689	0.64	0.765	0.078	71.299	15.493	0.373	0.358	7.758
	se	0.742	0.045	0.045	2.631	4.791	1.118	1.278	26.165	0.164	0.157	0.0056	10.74	1.82	0.036	0.026	3.261
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14208	0.822	*0.909	*81.378	*122.628	28.708	28.451	478.677	0.792	0.886	0.088	72.289	14.018	0.416	0.424	10.436
	se	0.742	0.044	0.044	2.598	4.73	1.104	1.262	25.835	0.161	0.155	0.0056	10.604	1.797	0.035	0.026	3.22

n = number of animals
LS mean = least square mean
se = standard error of least square mean
Significance level of comparison with control (0 mg/kg/day) using William's test * = p<0.05

Prostatitis was reported for 0/5 (0), 2/5(LD), 1/5(MD) and 2/5(HD) dogs and aspermatogenesis was reported for 1/5 (LD) dogs and 0 for all other groups. No drug-associated histopath findings were reported for the adrenal glands.

Plasma level drug determination showed that the AUC increased with increasing dose in a greater than linear fashion. Females showed slightly greater exposure than males at the HD.

Table 9
Ranolazine ; One Year Oral Toxicity Study in Dogs
Group Mean Summary of Pharmacokinetic Parameters

Dose (mg/kg/day)	Sex	6 Month Timepoint		12 Month Timepoint	
		C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)
10	Male Female	960 ± 401 429 ± 412	2997 ± 472 1687 ± 646	824 ± 587 1008 ± 1077	3108 ± 533* 3423 ± 2926**
25	Male Female	2621 ± 1856 2256 ± 824	8784 ± 2601 6864 ± 1990	2139 ± 1591 3505 ± 1762	8203 ± 3177* 8873 ± 3924
60	Male Female	8768 ± 3979 9716 ± 2811	33232 ± 9809 34796 ± 5016	6536 ± 2592 12816 ± 5688	29520 ± 12432 40409 ± 12199

Values are expressed in terms of ranolazine dihydrochloride.
n=5 except *n=4, **n=2.

The urinalysis was essentially qualitative. Given this, it does not appear that there were significant findings in the urinalysis data.

The only data provided from the ECGs was heart rate, shown below. It may be seen that average heart rate for the drug-treated males decreased at the 1 hour post-dose observation point. There was no pattern apparent in the data for the females.

Reviewer's summary of mean heart rates (beats/min) males

dose	Pre-trial	Day 1 (hours after dose)				Month 5 (hours after dose)			
		Pre-dose	1	6	24	Pre-dose	1	6	24
0	74	74	78	88	75	73	92	97	85
10	126	114	100	113	104	93	91	121	106
25	122	113	98	125	108	106	97	110	119
60	109	105	104	109	111	106	96	104	104

Summary of heart rates for males continued

dose	Pre-trial	Month 11 (hours after dose)			
		Pre-dose	1	6	24
0	74	98	112	107	104
10	126	111	105	129	126
25	122	113	102	112	119
60	109	111	99	110	129

Reviewer's summary of mean heart rates (beats/min) females

dose	Pre-trial	Day 1 (hours after dose)				Month 5 (hours after dose)			
		Pre-dose	1	6	24	Pre-dose	1	6	24
0	120	104	94	114	93	94	98	123	108
10	109	111	97	112	115	113	103	128	118
25	120	107	89	110	106	104	105	126	120
60	112	108	91	99	100	102	105	119	119

Summary of heart rates for females continued

dose	Pre-trial	Month 11 (hours after dose)			
		Pre-dose	1	6	24
0	120	108	107	123	102
10	109	129	123	143	123
25	120	118	112	133	126
60	112	121	123	134	130

Study title: RS-43285 RHT: Three month oral toxicity study in rats

Key study findings: Unscheduled mortality was seen at 5 (1f), 250 (2m) and 500 (3m, 4f) mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg), convulsions(≥250 mg/kg). The HD group also showed ptosis/sedation. The clinical chemistry findings at doses ≥ 250 mg/kg included decreased serum sodium, increased alkaline phosphatase and increased glucose. Absolute and relative liver and adrenal weight was increased at doses ≥50 mg/kg. Relative weight of the heart, pituitary, kidneys and spleen were also increased in both sexes at doses ≥250 mg/kg. Microscopic findings were reported for the HD only and included centrilobular hepatocyte enlargement, pulmonary alveolar foam cell proliferation and adrenal cortical cell vacuolation. Whether the adrenal effect is primary or secondary to effects on electrolytes cannot be determined from the available information. The urinalysis data was not provided nor was the complete pathology record made available as evidenced by textual references to findings in the 250 mg/kg group that were not presented in the pathology data. The manufacturing and stability data (Appendix I), noted that "placebo analysis indicated an RS-43285-193 content of less than 1.15×10^{-5} to less than 7.15×10^{-5} % w/w." It was not made clear if these are the limits of detection and quantitation or if ranolazine was present in the vehicle preparations.

Study no: AT3465, SS/012/85
Volume #, and page #: vol 16, p.224
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 1984
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: sodium hydroxide and distilled water, pH=4; vehicle was distilled water

Methods: Sprague-Dawley, CD rats (Charles River, Kent) 12 per sex per group given once daily oral doses of ranolazine at 0, 5, 50, 250 and 500 mg/kg/day for 91 days. Signs were evaluated daily around time of dosing. Body weight and food consumption were recorded weekly. Ophthalmoscopy was performed pre-treatment and during week 12. For hematology and clinical chemistry blood was collected from five animals per sex per group before the dosing phase. Ten rats per sex per group were sampled after 4,8 and 12 weeks of dosing. A four-hour urinalysis was performed for 5 animals per sex per group before dosing started and after 4,8, and 12 weeks of dosing. All animals surviving to scheduled necropsy were euthanized and examined. Specified organs were weighed and collected.

Results:
Unscheduled mortality: 1f @ 5mg/kg; 2m @ 250 mg/kg; 3m and 4f @500 mg/kg. No signs were reported for the lowest dose group. Clinical signs at 50 mg/kg were reported as infrequent and included salivation. Females given 250 mg/kg showed primarily salivation 10-30 minutes after dosing and sometimes prior to dosing. Males in this group were subdued and one also showed salivation and hunched posture. The HD animals showed salivation, subdued behavior, ptosis and mydriasis, prostration, convulsions and hyperventilation. Signs tended to resolve by approximately 6 hours after dosing. Signs were most evident in the first half of the study with females more affected than males, especially in weeks 1 and 2. The mydriasis and ptosis were usually seen in conjunction with convulsions, hyperventilation and in the majority of cases, death. Body condition and appearance was reported to deteriorate in the HD females.

HD males and females gained on average 21% and 8.8% less than their control groups respectively. Food consumption was somewhat decreased in the HD groups.

Ophthalmology: It was reported that there were no treatment-related effects. There was no evidence that a qualified ophthalmologist had made that decision or had evaluated the animals.

Hematology: There were some slight changes in hematology parameters that were marked as statistically significant. The biological significance, if any, is doubtful.

Clinical Chemistry: In both sexes, serum sodium was significantly decreased at 8 and 12 weeks in the two highest dose groups. Glucose was increased in the 2 highest dose groups of females as shown here. There were scattered changes in other parameters, but for the most part lacking in dose dependence or identifiable patterns.

Males Females

Time Point	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl
Pre-dose	All		Mean	139	5.3	11.0	85
			SD	1	0.5	2.4	10
4 Weeks	1	0	Mean	152	5.1	15.6	123
			SD	1	0.3	2.0	19
	2	5	Mean	151	5.0	16.1	124
			SD	1	0.2	2.2	12
	3	50	Mean	151	4.9	15.2	135
			SD	1	0.3	2.4	25
	4	250	Mean	149**	4.8	18.4	137
			SD	2	0.3	5.7	37
	5	500	Mean	148**	5.1	16.5	125
			SD	2	0.4	2.7	15
8 Weeks	1	0	Mean	147	5.5	16.7	161
			SD	1	0.8	2.9	28
	2	5	Mean	146	5.3	19.0	206
			SD	1	0.6	1.7	41
	3	50	Mean	146	5.0	18.4	164
			SD	1	0.5	1.5	52
	4	250	Mean	145**	5.1	19.3	176
			SD	2	0.4	6.7	35
	5	500	Mean	144**	5.4	18.5	167
			SD	1	0.9	3.1	42
12 Weeks	1	0	Mean	145	4.8	13.3	191
			SD	1	0.4	0.9	48
	2	5	Mean	147	4.9	13.5	168
			SD	2	0.4	1.9	51
	3	50	Mean	146	5.2	14.9	174
			SD	1	0.6	1.7	40
	4	250	Mean	146	5.5	16.0**	165
			SD	1	0.4	1.7	32
	5	500	Mean	145	4.7	16.0**	157
			SD	1	0.5	2.9	28

* p < 0.05
* p < 0.01

Time Point	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl
Pre-dose	All		Mean	139	4.9	15.5	101
			SD	1	0.4	1.8	13
4 Weeks	1	0	Mean	148	5.4	22.1	120
			SD	1	0.9	2.3	16
	2	5	Mean	149	4.9	20.4	153
			SD	2	0.7	2.8	37
	3	50	Mean	148	5.0	20.7	123
			SD	2	0.8	3.0	8
	4	250	Mean	147	4.8	22.7	149**
			SD	1	0.6	3.9	24
	5	500	Mean	147	4.3**	18.8*	207**
			SD	1	0.4	2.9	17
8 Weeks	1	0	Mean	149	5.2	21.1	161
			SD	1	0.6	3.1	28
	2	5	Mean	150	4.6	20.5	207
			SD	1	0.5	2.6	30
	3	50	Mean	149	4.8	22.1	161
			SD	0	0.7	3.8	19
	4	250	Mean	148*	5.3	17.9*	209**
			SD	1	0.4	2.0	33
	5	500	Mean	148*	4.6**	17.5*	210**
			SD	1	0.4	0.4	29
12 Weeks	1	0	Mean	145	5.3	19.8	137
			SD	1	0.6	2.8	38
	2	5	Mean	145	4.5	20.3	157
			SD	1	0.7	2.6	28
	3	50	Mean	144**	4.8	20.4	123
			SD	1	0.7	1.7	13
	4	250	Mean	143**	4.9	19.3	165*
			SD	1	0.8	2.6	28
	5	500	Mean	142**	4.7	18.7	168*
			SD	2	0.5	2.4	29

* p < 0.05
** p < 0.01

Alkaline phosphatase was increased in both sexes at the two highest doses.

Reviewer's summary of Alkaline Phosphatase Values.

timepoint	Dose mg/kg/day	Alkaline phosphatase (IU/l)	
		females	males
predose	all	128±26	214±31
4 weeks	0	65±17	95±18
	5	61±13	99±20
	50	65±15	102±20
	250	85*±19	109±31
	500	129**±58	150**±33
8 weeks	0	50±15	74±14
	5	50±15	73±16
	50	57±19	75±19
	250	65±24	85±17
	500	84**±25	111*±43
12 weeks	0	35±15	56±11
	5	29±9	55±12
	50	43±14	57±18
	250	55*±19	68±17
	500	63**±24	91**±35

Organ weights: absolute liver and adrenal weight was increased in both sexes at doses ≥ 50 mg/kg. Absolute spleen weight was increased in the females while kidney weight was increased in the males. Relative weight of the heart, pituitary, liver, kidneys, spleen and adrenals were increased in both sexes.

RS-43285 RHT : Three Month Oral Toxicity Study in Rats

Organ Weights as a Percentage of Bodyweight Group Summary

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary x1000	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Body Weight (g)
MALES														
1	0	Mean	0.45	0.32	1.22	3.46	3.01	0.21	0.70	0.18	0.014	0.08	6.07	491
		SD	0.05	0.02	0.12	0.49	0.21	0.06	0.08	0.03	0.002	0.01	1.32	42
2	5	Mean	0.48	0.32	1.14	3.28	2.98	0.22	0.69	0.19	0.017	0.09	6.65	490
		SD	0.09	0.02	0.22	0.44	0.25	0.07	0.04	0.02	0.003	0.02	0.82	35
3	50	Mean	0.47	0.32	1.25	3.21	3.11	0.22	0.72	0.19	0.018*	0.09	5.91	482
		SD	0.03	0.02	0.12	0.30	0.33	0.04	0.07	0.02	0.002	0.01	1.67	33
4	250	Mean	0.44	0.33	1.21	2.93**	3.52***	0.24	0.77**	0.20	0.024***	0.11***	6.34	502
		SD	0.05	0.02	0.14	0.47	0.32	0.05	0.07	0.02	0.005	0.02	1.14	47
5	500	Mean	0.49	0.39*	1.38*	3.66	3.99***	0.19	0.89***	0.23***	0.031***	0.09	7.66**	435
		SD	0.03	0.05	0.07	0.46	0.42	0.04	0.07	0.04	0.006	0.02	0.93	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary x1000	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Body Weight (g)
FEMALES														
1	0	Mean	0.71	0.38	0.057	6.42	3.33	0.30	0.80	0.21	0.030	0.12	9.16	277
		SD	0.06	0.03	0.010	1.09	0.54	0.07	0.09	0.03	0.006	0.03	1.56	23
2	5	Mean	0.72	0.40	0.062	6.96	3.17	0.31	0.80	0.21	0.030	0.13	8.34	283
		SD	0.07	0.03	0.010	1.15	0.31	0.06	0.06	0.03	0.006	0.02	1.61	22
3	50	Mean	0.75	0.41	0.067	7.83**	3.61	0.35	0.85	0.24	0.035	0.13	10.26	273
		SD	0.06	0.05	0.016	1.50	0.38	0.10	0.10	0.04	0.006	0.02	1.63	25
4	250	Mean	0.73	0.43*	0.072*	6.94	4.15***	0.31	0.86	0.26**	0.048***	0.15**	9.95	272
		SD	0.05	0.03	0.019	1.17	0.50	0.07	0.06	0.05	0.006	0.02	1.58	11
5	500	Mean	0.77**	0.44**	0.059	7.77*	4.54***	0.39*	0.91**	0.29***	0.067***	0.15*	10.01	248
		SD	0.04	0.03	0.009	0.85	0.52	0.10	0.10	0.05	0.010	0.03	2.05	17

* p 0.05
** p 0.01
*** p 0.001

TABLE 5

RS-43285 RPT : Three Month Oral Toxicity Study in Rats

Organ Weights Group Summary (g)

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Body-Weight (g)
MALES														
1	0	Mean	2.19	1.58	3.97	0.017	14.75	1.03	3.39	0.91	0.071	0.40	0.030	491
		SD	0.11	0.09	0.43	0.002	1.37	0.27	0.27	0.14	0.010	0.07	0.007	42
2	5	Mean	2.32	1.55	3.61	0.016	14.65	1.09	3.38	0.91	0.081*	0.46	0.033	490
		SD	0.32	0.10	1.12	0.002	1.78	0.31	0.22	0.11	0.014	0.10	0.005	35
3	50	Mean	2.25	1.55	4.01	0.015	14.96	1.08	3.46	0.89	0.085**	0.44	0.028	482
		SD	0.10	0.11	0.50	0.002	1.57	0.21	0.32	0.08	0.011	0.08	0.008	33
4	250	Mean	2.18	1.64	4.01	0.015**	17.65***	1.20	3.86***	1.02	0.122***	0.55***	0.032	502
		SD	0.08	0.12	0.39	0.002	2.03	0.29	0.26	0.18	0.025	0.10	0.006	47
5	500	Mean	2.12	1.70*	3.98	0.016	17.37***	0.85	3.85***	1.01	0.136***	0.41	0.033	435**
		SD	0.07	0.25	0.39	0.002	2.13	0.19	0.27	0.22	0.030	0.12	0.004	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Body-Weight (g)
FEMALES														
1	0	Mean	1.95	1.06	0.157	0.018	9.18	0.82	2.21	0.57	0.083	0.33	0.025	277
		SD	0.13	0.07	0.029	0.003	1.41	0.21	0.30	0.08	0.014	0.09	0.004	23
2	5	Mean	2.04	1.11	0.177	0.020	8.99	0.86	2.27	0.60	0.086	0.37	0.023	283
		SD	0.10	0.08	0.043	0.003	1.25	0.13	0.18	0.08	0.020	0.04	0.004	22
3	50	Mean	2.03	1.11	0.182	0.021**	9.82	0.96	2.31	0.64	0.096	0.34	0.028	273
		SD	0.10	0.13	0.039	0.004	1.17	0.22	0.20	0.10	0.013	0.07	0.005	25
4	250	Mean	1.99	1.16*	0.197*	0.019	11.30***	0.84	2.33	0.70**	0.130***	0.41*	0.027	272
		SD	0.14	0.09	0.057	0.003	1.43	0.18	0.18	0.12	0.017	0.05	0.004	11
5	500	Mean	1.91	1.08	0.146	0.019	11.30**	0.96	2.25	0.72**	0.166***	0.36	0.025	248***
		SD	0.10	0.11	0.022	0.002	1.64	0.24	0.25	0.12	0.028	0.09	0.005	17

* p < 0.05
** p < 0.01
*** p < 0.001

Necropsy/microscopic findings were reported only for the highest dose group of each sex. These are summarized below in the reviewer's table.

Reviewer's summary of microscopic findings

finding	Males	Females
Centrilobular hepatocyte enlargement	0/12(c), 1/12 hd	0/12(c), 7/12 hd
Pulmonary alveolar foam cell proliferation	1/12(c), 6/12 hd	0/12(c), 2/12 hd
Adrenal cortical vacuolation	0/12(c), ¾ (250 mg/kg), 12/12 hd	0/12(c), 7/12 hd
Adrenal cortical necrosis	0/0 (c), 0/0 (c)	0/0 (c), 1/12 hd

The sponsor states in the text (p.251) that adrenal cortical vacuolation occurred in 3/3 males in the 250 mg/kg group. In the tables of microscopic findings presented on pages 264-266, findings are presented for control and 500 mg/kg groups only. Individual animal pathology data was reported pages 312 to 339 but for fewer animals than were in each group. The data was not complete pathology records but selected findings. Was this the sum total of what was generated? Why was this not summarized in the incidence tables also?

Summary: The urinalysis data was not provided and it was not clear if the complete pathology data was provided. Unscheduled mortality was seen at 5, 250 and 500 mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg), convulsions (≥250 mg/kg). The clinical chemistry (decreased serum sodium and increased alkaline phosphatase) and microscopic findings (adrenal vacuolation) are supportive of adrenal involvement. Whether the adrenal effect is primary or secondary to effects on electrolytes cannot be determined from the available information. Hepatic enlargement was noted both in the organ weight data and in the microscopic findings. Another finding of note was an increased incidence of pulmonary alveolar foam cell proliferation. A complete assessment of the drug-associated effects cannot be made from the report.

Study title: RS43285 RJT: Six month oral toxicity study in rats with a one month recovery period

Key study findings:
Clinical signs were reported for doses of ≥50 mg/kg/day and included the salivation, sedation, prostration, tachypnea or shallow breathing, hunched appearance, ataxia, piloerection and convulsions noted in other studies. Signs were usually present within 1-2 hours after dosing and showed partial to complete resolution by 4-6 hours post-dose. Target cells, reported in the hematology results, may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were poorly presented and not susceptible to easy interpretation. However, adrenal organ weights and histopathology support the adrenal gland as a target organ as well as alterations in liver function and possibly cardiac muscle damage.

Study no: AT3935, SS//047/87
Volume #, and page #: vol 18., p.3
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August 1986
GLP compliance: statement included
QA report: yes () no (x)
Drug, lot #, radiolabel, and % purity: lot number 15 aka 153SS0785
Formulation/vehicle: control was dH_2O

Methods: Sprague-Dawley CD rats (Charles River, Kent) were assigned to groups with 30 males and 30 females in the control and HD groups and 20 males and females in each of the LD and MD groups. All rats were dosed for at least 182 days by oral gavage. At the end of the dosing period, all LD and MD animals and the first 20 rats in the control and HD groups were

euthanized. The remaining 10 rats in the control and HD groups were given a 30 day drug-free recovery period. Doses used were 0,5,50 and 200 mg/kg.

Blood for determination of plasma level drug values was taken at one, three and six months from animals not sampled for clinical pathology (approximately 10/sex/group). Clinical signs were monitored each day. Bodyweight and food consumption were recorded weekly. Ophthalmoscopy was performed before dosing started and again in weeks 12 and 25. Ten animals per sex per group were sampled for clinical pathology before dosing, weeks 4, 13 and 25 of dosing. Urinalysis was performed on samples obtained over a 4 hour period in metabolism cages (8 animals per sex per group) before dosing started and again at 4,13 and 25 weeks of dosing.

All animals that died on study were examined as were the animals surviving to scheduled termination. The latter group of animals were euthanized 24 hours after the last dose. Organ weights and microscopic evaluation were made for a specified list of tissues. Adrenals from all recovery group animals were examined. The sponsor stated on p. 22 that " As treatment-related changes at the terminal kill were confined to the adrenals and liver Oil-Red-O, histopathological examination of altered tissue in the recovery groups was considered unnecessary."

Liver samples were taken at euthanasia from 8 animals/sex for the control and MD groups. Hepatic levels of CYP450 and microsomal protein were reported separately. Adrenals from all recovery group animals were examined. The sponsor states

Recovery Groups
Adrenals from all recovery group animals in groups 1 and 4 were examined. As treatment-related changes at the terminal kill were confined to the adrenals and liver Oil-Red-O, histopathological examination of altered tissue in the recovery groups was considered unnecessary.

Results: One LDf and 3HDm died or were euthanized in extremis. Clinical signs included salivation, seen within 10 minutes of dosing in the MD and HD groups. Signs in the HD group included subdued behavior, hunched appearance, ataxia, prostration, tachypnea, piloerection, ptyalism and convulsions. Signs usually occurred within 1-2 hours of dosing with partial to complete resolution of signs 4-6 hours post-dose.
Bodyweights: baseline values were not provided. It does appear that in the first month the treated rats gained less than the control group. The sponsor's table is shown here.

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Group Mean Bodyweights (g) - Day 1 and 24 Weeks - Groups 1 and 4

Group No./Sex	Dose mg/kg/day	Sub-Group	Day 1	24 Weeks
MALES				
1	0	Non-recovery	257	590
		Recovery	252	602
4	200	Non-recovery	256	605
		Recovery	251	533
FEMALES				
1	0	Non-recovery	186	326
		Recovery	188	319
4	200	Non-recovery	185	318
		Recovery	175	309

Food consumption appeared to be comparable between the groups.
Hematology: Target cells were reported for the HD animals of both sexes (7/10 males, 4/10 females) at 25 weeks. The sponsor suggests this as a cause for the slight increase in MCV. Neutrophil counts were consistently higher in drug-treated males compared to the control group. The same trend was not apparent in the females.

Reviewer's summary of neutrophil counts x 10^9/l in male rats

Dose group Mg/kg	Time point				
	pre	4weeks	13weeks	25 weeks	Recovery
0	1.2±0.5	1.9±0.6	2.7±0.9	2.4±1.0	2.3±0.6
5		2.5±1.1	2.6±1.8	4.1±4.6	
50		2.8±1.3	2.3±1.3	3.1±1.2	
200		3.2*±1.0	3.4±3.1	4.4±2.6	2.8±0.9

Clinical chemistry: in both sexes, Na, K, glucose and cholesterol were affected. Of concern also is that at the HD in both sexes, LDH and HBDH were also elevated. The elevation of CK, LDH and HBDH together is suggestive of cardiac muscle damage. While CK was not measured, the consistent elevation of LDH and HBDH in the HD, both sexes, is noteworthy.

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Clinical Chemistry Group Summary - Males

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
25	1	0	Mean	144	4.9	32.4	155	23	41	125	36	96	91	115	0.8
			SD	1	0.4	4.6	29	5	5	38	10	18	11	46	0.3
	2	5	Mean	144	5.1	30.6	155	24	38	122	36	96	101	112	0.9
			SD	1	0.5	2.6	39	6	5	23	8	26	19	47	0.3
	3	50	Mean	146***	5.0	31.6	129	24	39	137	38	109	107	108	1.0
			SD	1	0.4	2.9	35	5	8	24	6	20	27	42	0.3
	4	200	Mean	144	5.3	31.0	136	23	38	191**	51**	106	123**	108	0.8
			SD	1	0.3	4.6	32	6	5	50	13	34	24	32	0.2
4 Recovery	1	0	Mean	146	5.2	29.8	124	27	31	81	26	93	103	171	1.2
			SD	1	0.4	4.6	15	7	4	32	9	36	17	63	0.3
	4	200	Mean	145	5.2	34.0	159	24	32	87	28	112	94	172	1.0
			SD	1	0.4	4.0	42	5	3	47	13	29	27	107	0.4

** p less than 0.01
*** p less than 0.001

Clinical Chemistry Group Summary - Males

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
Pre-dose	All	-	Mean	144	5.2	25.9	97	21	44	103	31	367	118	108	1.2
			SD	1	0.5	5.2	18	4	4	33	9	73	21	36	0.2
4	1	0	Mean	152	5.0	35.0	146	21	38	113	32	215	92	101	1.3
			SD	1	0.4	3.8	37	3	3	53	14	30	13	27	0.2
	2	5	Mean	152	5.0	33.4	123	23	40	110	31	202	100	97	1.5
			SD	1	0.4	3.1	16	3	4	25	7	45	13	41	0.2
	3	50	Mean	152	4.9	34.9	115*	23	38	129	35	217	99	81	1.5
			SD	1	0.3	4.0	18	4	2	62	15	44	17	18	0.2
	4	200	Mean	151**	5.1	33.3	125*	25*	40	140	37	206	123**	93	1.5
			SD	1	0.4	3.1	17	5	2	22	6	47	22	33	0.3
13	1	0	Mean	147	5.0	37.0	124	22	43	111	33	124	92	97	0.9
			SD	2	0.3	7.0	22	4	11	33	10	18	11	41	0.2
	2	5	Mean	146	5.2	34.2	131	21	38	122	34	109	95	91	0.9
			SD	1	0.4	2.4	22	3	3	39	11	26	16	51	0.2
	3	50	Mean	149*	4.9	35.7	119	20	36	120	32	121	98	79	1.0
			SD	1	0.2	4.1	26	3	3	23	6	19	23	23	0.2
	4	200	Mean	148*	5.1	32.8	119	21	38	137	37	123	121**	94	1.1
			SD	2	0.3	3.1	18	5	2	49	12	33	21	33	0.2

* p less than 0.05
** p less than 0.01

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Clinical Chemistry Group Summary - Females

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
Pre-dose	All	-	Mean	141	5.1	35.5	93	16	42	173	48	246	99	57	0.8
			SD	1	0.5	5.3	14	2	4	58	15	44	20	15	0.2
4	1	0	Mean	146	5.3	37.3	107	20	40	128	35	112	90	56	1.4
			SD	2	0.5	3.7	12	2	4	41	14	14	11	20	0.4
	2	5	Mean	147	4.8*	36.5	107	24	43	124	34	116	103	58	1.5
			SD	1	0.5	4.5	12	8	9	51	14	21	11	20	0.2
	3	50	Mean	147	4.9	40.1	105	28	42	106	30	121	118**	62	1.2
			SD	2	0.5	5.2	13	21	13	30	9	23	22	15	0.4
	4	200	Mean	145	4.6**	43.0**	130	29**	41	190*	51*	125	157**	59	1.1
			SD	1	0.5	4.6	44	6	5	92	23	23	27	17	0.3
13	1	0	Mean	148	4.8	37.7	113	22	42	113	34	58	99	72	1.2
			SD	1	0.5	5.5	17	9	13	30	8	14	16	17	0.2
	2	5	Mean	147	5.0	39.0	103	28	41	109	32	61	112	73	1.2
			SD	1	0.4	9.5	8	15	9	35	9	10	11	18	0.2
	3	50	Mean	148	4.8	35.3	116	18	35	102	30	55	125**	76	1.2
			SD	1	0.3	3.9	23	5	3	20	5	10	22	18	0.2
	4	200	Mean	148	5.1	36.7	147*	22	33*	148	42	63	164**	79	1.2
			SD	1	0.6	5.5	38	5	4	79	20	14	23	26	0.5

* p less than 0.05
** p less than 0.01

TABLE 4

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Clinical Chemistry Group Summary - Females

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
25	1	0	Mean	140	5.1	34.9	122	42	59	124	35	39	122	95	n/a
			SD	1	0.5	4.3	21	25	35	12	8	8	17	33	
	2	5	Mean	140	4.6*	36.8	125	73	84	126	35	51*	138	104	1.1
			SD	1	0.5	7.3	23	50	49	34	9	13	34	31	0.5
	3	50	Mean	140	4.3*	33.3	133	44	50	113	32	48*	146*	110	1.0
			SD	1	0.5	4.0	30	37	23	26	7	10	24	22	0.3
	4	200	Mean	138**	4.7*	36.1	134	42	42	153	37	47*	198**	129	1.1
			SD	1	0.7	1.8	22	31	18	97	23	9	29	65	0.5
4 Recovery	1	0	Mean	143	5.1	36.7	169	50	52	126	38	59	150	168	1.4
			SD	1	0.5	4.4	28	20	20	64	17	23	27	41	0.4
	4	200	Mean	143	5.1	33.6	131	33	38	121	36	46	137	204	1.4
			SD	1	0.5	4.4	29	18	11	54	14	19	20	117	0.4

n/a - Mean and SD not applicable. Sample size equal to 3

* p less than 0.05
** p less than 0.01

Ophthalmoscopy and plasma drug levels were not presented. The report does not specify who actually performed the ophthalmic exam, that is, was it a staff veterinarian or a veterinary ophthalmologist?

Urinalysis data was presented for single animals in a form that did not lend itself to easy interpretation. Single letter abbreviations were used to indicate the parameter being measured with no key. Despite this, there appeared to be a slight increase in ketones, bile pigments, blood pigments and urobilinogen in the HD males at 13 and 25 weeks. There was also an increase in the incidence and degree of changes in the "A" column. The HD females showed a slight increase in urinary bile pigments.

The sponsor reports gross adrenal enlargement in 1/20 MDf, 1/20 HDm, 9/20HDf.
Organ weights: liver, adrenal, heart and kidney weights were increased in both sexes, with the effects more pronounced in the females. This is summarized in the reviewer's table below.

Reviewer's summary of organ weight data

Males : dose mg/kg/day				
	0	5	50	200
adrenal	0.063±0.01	0.065±0.01	0.065±0.011	0.089**±0.016
heart	1.65±0.18	1.75±0.18	1.75±0.28	1.78±0.25
Kidney	3.73±0.40	3.50±0.53	3.75±0.40	4.03±0.52
Liver	16.23±2.96	15.42±2.16	16.08±2.11	17.66±2.71
Females: dose mg/kg/day				
adrenal	0.074±0.011	0.071±0.012	0.083±0.019	0.108**±0.019
heart	1.13±0.11	1.04*±0.11	1.11±0.09	1.22**±0.13
Kidney	2.35±0.30	2.22±0.22	2.33±0.24	2.58*±0.33
Liver	9.48±1.09	9.08±0.95	9.99±1.22	11.04**±1.51
spleen	0.54±0.06	0.54±0.08	0.59*±0.08	0.65**±0.07
pituitary	0.017±0.004	0.017±0.003	0.020*±0.004	0.021**±0.004

*p<0.05, **p<0.01

There were no apparent organ weight effects in the recovery males. Liver and adrenal weight was still increased in the females at the end of the recovery period.

Reviewer's summary of end of recovery weights for females

	Control group at recovery end	HD at recovery end
Adrenal gland	0.067±0.017	0.082*±0.011
liver	8.92±0.63	9.64±1.23

*p<0.05

The only microscopic findings of apparent significance concerned the adrenal gland.
Vacuolation was reported only for males. Cytoplasmic foaminess was reported only for females.
This is summarized in the reviewer's table below.

Reviewer's summary of adrenal gland findings

	Dose group (mg/kg/day)			
	0	5	50	200
Diffuse vacuolation of the zona fasiculata (males only)	0/20	6/20	9/20	18/18
Diffuse cytoplasmic foaminess of the zona fasiculata (females only)	0/20	0/20	0/20	17/20

Of the 4 unscheduled deaths (p.32), 3 showed moderate/marked diffuse cytoplasmic vacuolation of the fasciculata cells. These 3 animals included a recovery male. The female was the1/4 with no histologic evidence of change. The sponsor also notes that these animals were not tabulated. In the recovery males, vacuolation of the zona fasciulata was still present in all HD animals but was "reduced." In one animal the findings were "marked."

Summary: Target cells may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were poorly presented and not susceptible to easy interpretation. However, adrenal organ weights and histopathology support the adrenal gland as a target organ as well as alterations in liver function and possibly cardiac muscle damage. In the Discussion section (p.36), the sponsor states that:

> The normal foamy appearance of cells of the zona fasciculata is due to the presence of lipid which may serve as a precursor of steroid biosynthesis. The vacuolation and increased foamy appearance of these cells after administration of RS 43285 is the probable result of accumulation of cytoplasmic lipid. Similar changes have been observed with a number of compounds which inhibit steroidogenesis[9,10]. However, in contrast to such compounds as aminoglutethimide, amphenone and other corticosteroid inhibitors administration of RS 43285 for six months does not appear to induce cortical cell degeneration, atrophy or necrosis[10]. Following the one month recovery period the effect was diminished in top dose males and was no longer evident in top dose females.

> Although the changes in these rat adrenals may be representative of a functional disturbance in the cells of the zona fasciculata it should be emphasised that no firm evidence of adrenocortical insufficiency was observed even at the highest dose level in this six month study with RS 43285.
>
> It may be concluded that oral administration of RS 43285 for six months produced no adverse effects in female rats at 5 mg/kg/day. Evidence of altered activity in adrenal cells was observed in male rats at 5 mg/kg/day. However the changes are reversible and may be attributable to the pharmacological activity of the compound.

The PK data for this study was not located in the submission. A study conducted in 1994 used doses of 2,5, 50 and 150 mg/kg daily for 6 months (AT6811). Doses of ≥50 mg/kg/day showed a tendency towards increasing plasma exposure over time.

Summary of comparable pk

	Females 150 mg/kg/day			Males 150 mg/kg/day		
	Rat AUC_{0-24} ng.hr/ml	Human AUC_{0-24}	Rel exposure	Rat AUC_{0-24} ng.hr/ml	Human AUC_{0-24}	Rel exposure

Day 1	99800	Tid dosing	1.48X	59500	33700	0.89
Day 92	126000	33700	1.87	63600		0.95
Day 183	167000		2.48	127000		1.89

While the sponsor identifies a NOEL for females at 5 mg/kg and for males at 2mg/kg, this does not equal the therapeutic exposure that a human could expect to achieve.

Study title: One year oral toxicity study in rats

Key study findings: Signs seen ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male.

The HD males had gained on average 15% less body weight than the control group ($p<0.05$ by Student's t test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups.
Both sexes at the HD showed slight decreases in Hb and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. The adrenal gland was again a target organ in the females. A drug effect was seen in increased incidences of pneumonia (described by the sponsor as inhalational) in both sexes of drug-treated animals. The sponsor hypothesized the possibility of a long-term effect on pharyngeal/esophageal muscle tone.

Study no: AT6544/ SS/003/93
Volume #, and page #: vol 28, 113
Conducting laboratory and location: Syntex Research, Edinburgh, Scotland
Date of study initiation: November 5, 1991
GLP compliance:
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle: aqueous buffered solution. Water was used as the vehicle control

Methods Male and female rats (Crl:CD(SD)BR), 20/sex/group were orally gavaged once a day for 1 year with 0,20,50 or 200 mg/kg/day ranolazine. Rats were observed daily for signs. Body weights were recorded weekly and food consumption was estimated weekly. Blood was collected for clinical pathology pre-test and after 4, 8 and 12 months of dosing. Blood for plasma level determination of drug was collected after 6 and 12 months of dosing. Samples were collected from 10 rats/sex/group. Those not sampled for clinical pathology were sampled for plasma level drug determination at 30 mins., 1.5,3,6 and 24 hours after dosing. Urinalysis was performed at similar timepoints. At time of necropsy, tissues were collected from all animals but examined only from the HD, control groups and premature decedents. Lungs and adrenals were examined from all animals.

Results: Four animals were euthanized (1FC, 2 M 2 mg/kg, 1F 50 mg/kg) were euthanized due to the size of SQ masses. 12 animals were found dead with little to no reported signs: 2m/2f (controls); 1m (20 mg/kg);1m/1f (50 mg/kg); 4f/1m (200 mg/kg). 10 more animals were euthanized due to marked clinical signs including hunched appearance, weight loss, labored or irregular breathing, convulsion, piloerection and subdued behavior. The total number of unscheduled mortalities was summarized by the sponsor as shown below:

Summary of deaths

Dose of ranolazine (mg/kg/day)	Number of animals M/F	Deaths M/F
0	20/20	4/3
2	20/20	2/0
20	20/20	1/0
50	20/20	1/3
200	20/20	5/7

Signs seen at doses ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male.

By week 52, the HD males had gained on average 15% less body weight than the control group ($p<0.05$ by Student's t test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups.

Hematology: Both sexes at the HD showed slight decreases in Hb and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. From the text of the report it was noted that an eosinophilia was apparent in 5 HD animals and 1 MD male at week 17. It was also noted that increased numbers of eosinophils were more apparent in the males at MD and HD. At week 33, the sponsor states that "…although only one male receiving 50 mg/kg/day and one male receiving 200 mg/kg/day recorded an eosinophilia, increased numbers of eosinophils continued to be seen. No obvious increase in granulocytes was apparent throughout the study."

Table 3
Ranolazine One Year Oral Toxicity Study in Rats
Haematology Group Summary - Females

Study Week	Group No	Dose mg/kg/day		Hb g/dl	RBC 10^{12}/l	HCT ratio	RBC Indices MCH pg	RBC Indices MCHC g/dl	RBC Indices MCV fl	Retics %	Plats 10^9/l	PT sec	PTT sec
0	All	0	Mean	14.4	7.07	0.458	20.3	31.4	64.8	8.6	970	-	-
			SD	0.9	0.49	0.027	0.6	0.4	1.8	1.2	119	-	-
17	1	0	Mean	15.3	8.24	0.471	18.7	32.6	57.2	1.8	800	19.0	20.0
			SD	0.2	0.32	0.010	0.8	0.4	2.2	0.4	117	1.5	2.0
	2	2	Mean	15.6	8.29	0.480	18.8	32.5	57.9	1.3	839	19.7	20.5
			SD	0.5	0.27	0.016	0.4	0.5	1.4	0.5	118	2.8	3.3
	3	20	Mean	15.2	8.03	0.474	18.9	*32.0	*59.1	1.5	753	19.1	21.1
			SD	0.6	0.26	0.017	0.6	0.6	1.8	0.5	88	2.3	4.0
	4	50	Mean	15.2	8.04	0.473	18.9	*32.2	*58.8	1.5	904	21.2	22.7
			SD	0.6	0.40	0.020	0.4	0.4	1.3	0.4	112	3.1	3.6
	5	200	Mean	14.9	**7.79	0.466	19.1	*32.0	**59.8	1.8	**1016	19.7	*24.3
			SD	0.7	0.30	0.020	0.4	0.6	1.4	0.4	79	1.6	4.0
33	1	0	Mean	15.1	8.32	0.479	18.2	31.5	57.6	1.2	781	23.7	24.8
			SD	0.5	0.30	0.017	0.6	0.4	2.1	0.3	79	1.0	1.0
	2	2	Mean	15.6	8.54	0.494	18.3	31.7	57.8	1.1	777	21.0	26.9
			SD	0.5	0.43	0.017	0.5	0.3	1.4	0.2	109	0.0	5.1
	3	20	Mean	15.3	8.35	0.490	18.3	31.2	58.7	1.2	742	23.6	27.4
			SD	0.9	0.50	0.028	0.6	0.3	1.9	0.2	124	3.4	3.0
	4	50	Mean	15.4	8.42	0.491	18.4	31.5	58.4	1.4	777	27.9	26.2
			SD	0.9	0.50	0.028	0.3	0.5	1.6	0.3	203	7.6	3.1
	5	200	Mean	14.8	8.03	0.481	18.5	**30.9	**58.9	**1.7	*915	25.3	**34.6
			SD	0.8	0.39	0.024	0.3	0.5	1.3	0.2	120	3.6	4.1
49	1	0	Mean	14.9	8.18	0.482	18.3	31.0	58.9	1.5	726	20.2	20.8
			SD	0.6	0.33	0.021	0.6	0.4	1.9	0.5	98	4.2	4.1
	2	2	Mean	15.5	8.49	0.499	18.3	31.1	58.8	1.0	715	18.1	19.3
			SD	0.6	0.39	0.019	0.4	0.4	1.6	0.4	94	1.6	2.5
	3	20	Mean	15.3	8.33	0.501	18.4	30.6	60.2	1.5	672	19.2	20.0
			SD	0.7	0.42	0.021	0.6	0.5	1.8	0.8	111	1.4	1.7
	4	50	Mean	15.1	8.17	0.487	18.5	30.9	59.8	1.6	728	18.5	20.5
			SD	1.1	0.67	0.033	0.4	0.4	1.6	0.6	135	0.6	2.4
	5	200	Mean	14.4	7.76	0.468	18.6	30.8	60.3	1.7	793	**17.3	19.2
			SD	1.0	0.60	0.032	0.3	0.4	1.5	0.5	134	1.8	2.3

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01

Table 3
Ranolazine One Year Oral Toxicity Study in Rats
Haematology Group Summary - Males

Study Week	Group No	Dose mg/kg/day		Hb g/dl	RBC 10^{12}/l	HCT ratio	RBC Indices MCH pg	RBC Indices MCHC g/dl	RBC Indices MCV fl	Retics %	Plats 10^9/l	PT sec	PTT sec
0	All	0	Mean	13.2	8.40	0.432	20.7	30.6	67.6	10.6	760	-	-
			SD	0.6	0.30	0.018	0.7	0.4	2.0	1.8	104	-	-
17	1	0	Mean	14.8	8.31	0.460	17.8	32.1	55.4	2.7	876	20.8	20.9
			SD	0.7	0.47	0.020	0.7	0.2	2.1	0.9	199	1.6	2.6
	2	2	Mean	15.0	8.59	0.467	17.5	32.2	54.3	1.9	890	21.4	20.8
			SD	0.6	0.33	0.014	0.3	0.4	0.9	0.7	121	1.1	1.4
	3	20	Mean	15.0	8.27	0.465	18.1	32.2	56.2	1.7	848	21.4	20.2
			SD	0.5	0.34	0.011	0.6	0.5	1.9	0.8	189	1.7	1.9
	4	50	Mean	14.8	8.53	0.464	17.4	32.0	54.5	2.0	900	21.7	21.4
			SD	0.5	0.37	0.018	0.6	0.4	1.9	0.5	168	2.9	2.5
	5	200	Mean	14.3	8.24	0.451	17.3	**31.6	54.9	2.9	*1081	21.0	22.6
			SD	0.9	0.65	0.026	0.7	0.6	2.4	1.6	192	3.4	6.4
33	1	0	Mean	15.0	8.84	0.487	17.0	30.8	55.1	1.1	839	26.5	23.1
			SD	0.4	0.38	0.016	0.7	0.6	2.3	0.2	124	5.5	4.3
	2	2	Mean	15.0	8.86	0.485	17.0	30.9	54.8	1.2	883	29.8	29.6
			SD	0.6	0.28	0.013	0.3	0.5	0.9	0.3	152	8.2	9.9
	3	20	Mean	14.9	8.51	0.482	17.6	31.0	56.6	1.2	948	30.9	29.1
			SD	0.5	0.33	0.013	0.5	0.4	1.6	0.4	129	7.3	7.5
	4	50	Mean	14.9	8.73	0.478	17.0	31.1	54.8	1.4	945	36.4	32.8
			SD	0.4	0.31	0.014	0.6	0.5	2.1	0.3	96	4.0	2.9
	5	200	Mean	14.4	8.48	*0.468	17.1	30.9	55.4	**1.7	**1143	30.5	*28.8
			SD	1.0	0.83	0.031	1.0	0.5	2.8	0.8	227	6.4	3.9
49	1	0	Mean	15.5	9.23	0.512	16.8	30.2	55.5	2.1	836	20.3	17.8
			SD	0.6	0.35	0.019	0.7	0.5	2.2	0.5	249	2.1	2.5
	2	2	Mean	15.1	9.13	0.503	16.8	30.1	55.0	1.9	941	19.4	17.4
			SD	0.5	0.33	0.014	0.3	0.5	1.0	0.5	159	1.1	1.3
	3	20	Mean	15.0	8.67	0.496	17.3	30.2	57.2	1.8	943	20.7	18.2
			SD	0.6	0.36	0.014	0.6	0.5	1.8	0.5	150	1.4	2.1
	4	50	Mean	15.3	9.11	0.505	16.8	30.3	55.5	2.1	983	19.7	18.2
			SD	0.4	0.35	0.013	0.7	0.4	2.4	0.4	155	1.3	1.9
	5	200	Mean	*14.7	8.85	0.493	16.6	29.8	55.8	2.5	**1087	22.0	19.4
			SD	1.2	0.70	0.035	0.6	0.8	2.1	1.0	179	3.6	4.4

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01

Table 4
Ranolazine : One Year Oral Toxicity Study in Rats
Clinical Chemistry Group Summary - Males

Study Week	Group No	Dose mg/kg/day		Na mmol/l	K mmol/l	Urea mmol/l	Gluc mmol/l	Chol mmol/l	Trig mmol/l	Creat µmol/l	ALT IU/l	AST IU/l	LDH IU/l	HBDH IU/l	Prot g/l
0	All	0	Mean	140	5.78	6.1	9.9	2.6	1.5	43	66	55	109	33	57
			SD	1	0.39	0.7	1.1	0.4	0.6	3	9	5	43	11	2
17	1	0	Mean	143	4.42	5.8	10.9	2.8	1.3	57	42	48	98	25	69
			SD	1	0.40	0.6	2.4	0.6	0.3	4	12	15	36	9	4
	2	2	Mean	144	4.36	5.9	10.7	2.7	1.3	+50	38	44	90	24	70
			SD	1	0.45	0.9	1.5	0.6	0.5	3	8	10	25	6	4
	3	20	Mean	143	4.22	6.2	12.4	2.6	1.4	59	39	46	101	27	70
			SD	1	0.47	0.8	0.8	0.4	0.5	3	12	13	32	7	3
	4	50	Mean	**144	4.11	6.2	13.0	2.4	1.4	+61	32	38	89	24	71
			SD	1	0.35	0.7	1.0	0.6	0.6	3	4	6	28	7	3
	5	200	Mean	**144	4.34	*7.1	12.1	+3.5	**2.0	60	34	42	106	29	**73
			SD	1	0.40	2.5	1.6	1.1	0.7	5	17	19	47	12	3
33	1	0	Mean	146	4.53	5.2	10.8	2.8	1.5	51	52	54	99	28	71
			SD	1	0.52	0.7	2.1	0.9	0.3	3	30	27	22	5	3
	2	2	Mean	145	4.73	5.2	+9.4	2.9	1.5	54	55	60	138	+38	71
			SD	3	0.57	0.8	0.8	0.8	0.8	2	25	25	45	11	3
	3	20	Mean	145	4.48	5.4	10.5	2.8	1.4	53	46	55	129	35	71
			SD	1	0.47	0.5	1.7	0.6	0.5	3	14	17	37	6	2
	4	50	Mean	145	4.84	5.4	+9.0	2.7	2.0	53	39	44	152	30	72
			SD	2	0.37	0.5	1.3	0.9	1.0	3	9	11	131	10	2
	5	200	Mean	146	4.77	6.8	10.6	+3.6	*2.4	+55	@30	39	108	31	73
			SD	1	0.37	3.2	1.8	1.7	0.9	4	4	10	49	12	3
49	1	0	Mean	146	4.50	5.0	9.6	3.3	1.6	53	55	60	83	28	71
			SD	1	0.50	0.7	2.1	1.0	0.5	3	29	28	30	10	4
	2	2	Mean	146	4.67	5.1	9.3	3.2	1.7	+57	36	47	+157	+48	71
			SD	2	0.51	0.5	1.6	1.0	0.5	2	6	9	130	35	4
	3	20	Mean	**144	4.33	5.5	9.9	3.3	1.6	55	46	55	129	40	71
			SD	0	0.49	0.8	1.7	0.8	0.5	2	15	14	35	9	2
	4	50	Mean	**145	4.77	5.4	8.8	3.4	2.4	55	36	46	134	40	72
			SD	1	0.44	0.8	1.5	1.4	1.1	3	12	14	55	16	2
	5	200	Mean	**144	4.36	**6.1	10.2	3.8	1.8	++57	@@27	39	134	40	73
			SD	1	0.60	0.7	1.9	1.0	0.5	4	6	10	62	18	3

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01
Student's t-test + = p<0.05, ++ = p<0.01 Shirley's test @ = p<0.05, @@ = p<0.01

Slight changes in the differential were marked as statistically significant but are of questionable biological significance.

Clinical chemistry showed slight decreases in serum sodium (m+f), increased cholesterol (m+f) and triglycerides for the HD males and increased creatinine concentrations for the drug-treated males. Simultaneous increases in LDH and HDBH were noted in the drug-treated males also.

Table 4
Ranolazine : One Year Oral Toxicity Study in Rats
Clinical Chemistry Group Summary - Females

Study Week	Group No	Dose mg/kg/day		Na mmol/l	K mmol/l	Urea mmol/l	Gluc mmol/l	Chol mmol/l	Trig mmol/l	Creat µmol/l	ALT IU/l	AST IU/l	LDH IU/l	HBDH IU/l	Prot g/l
0	All	0	Mean	142	4.96	6.9	9.3	2.5	1.4	45	43	43	103	30	63
			SD	1	0.40	1.0	1.0	0.4	0.4	3	7	4	30	8	3
17	1	0	Mean	143	4.57	5.7	9.0	2.2	1.5	64	39	51	113	31	71
			SD	1	0.28	0.7	1.6	0.5	0.6	5	12	24	43	10	3
	2	2	Mean	143	4.37	5.9	10.1	2.5	1.4	63	54	55	115	33	70
			SD	1	0.32	0.6	3.1	0.7	0.7	4	21	13	33	8	3
	3	20	Mean	143	4.26	*6.4	10.4	2.6	2.0	62	39	46	117	33	70
			SD	1	0.54	0.7	1.8	0.4	0.7	5	7	13	44	11	6
	4	50	Mean	143	4.47	*6.4	8.9	2.5	1.5	63	37	42	118	33	71
			SD	1	0.41	0.8	1.4	0.3	0.8	2	4	5	45	10	2
	5	200	Mean	144	4.27	**6.8	10.1	**3.8	1.2	64	34	38	123	34	71
			SD	1	0.37	0.8	2.0	0.7	0.5	5	8	11	36	9	4
33	1	0	Mean	149	4.97	6.3	8.8	2.8	2.0	62	49	61	145	40	75
			SD	2	0.54	0.9	1.8	0.5	0.7	3	16	21	43	10	4
	2	2	Mean	151	4.75	6.1	8.9	2.9	1.8	63	66	92	190	50	75
			SD	3	0.60	0.8	2.3	1.2	1.1	4	23	31	59	14	5
	3	20	Mean	149	++4.23	6.6	10.6	3.0	2.8	63	45	60	158	42	75
			SD	3	0.23	0.7	1.5	0.3	1.8	3	16	29	97	24	7
	4	50	Mean	150	4.70	6.6	8.9	3.0	1.8	64	48	59	167	45	77
			SD	1	0.43	1.2	1.1	0.6	0.8	4	13	24	63	16	4
	5	200	Mean	*147	4.70	6.4	9.5	**4.5	1.8	61	36	45	177	47	75
			SD	2	0.59	0.9	0.8	1.4	1.0	3	18	26	73	18	4
49	1	0	Mean	146	4.13	6.5	10.0	3.2	2.5	57	54	71	114	35	78
			SD	2	0.33	0.9	1.8	0.5	1.3	3	18	29	46	12	7
	2	2	Mean	145	4.45	6.0	9.8	3.3	2.5	58	58	85	148	46	78
			SD	1	0.86	1.1	2.4	1.7	1.4	3	20	41	90	23	5
	3	20	Mean	+145	4.11	7.2	10.0	3.1	2.2	60	45	59	115	34	79
			SD	1	0.43	1.1	1.5	0.3	0.8	5	18	25	64	17	4
	4	50	Mean	++144	4.22	6.3	8.7	3.7	2.3	59	40	56	107	34	82
			SD	1	0.29	0.8	1.6	0.7	1.2	1	16	27	40	11	5
	5	200	Mean	146	4.32	6.1	9.0	**5.8	2.0	+54	*37	*41	68	22	78
			SD	2	0.50	0.5	1.8	2.1	0.7	4	13	18	26	7	5

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01
Student's t-test + = p<0.05, ++ = p<0.01

A brief statement indicates that there were ophthalmic findings of corneal pitting, cataracts and corneal opacities. An ophthalmologist's report was not located. The sponsor felt the findings were unrelated to drug.

The absolute and normalized adrenal weights were increased in males and females at >50 mg/kg. Absolute and normalized liver and kidney weights were increased in both sexes at the HD.

Group Mean Organ weights adjusted for terminal body weights: males

Dose (mg/kg/day)		Terminal Bodyweight	Adrenal#	Brain	Heart	Kidney	Liver
0	n	16	15	15	16	16	16
	mean	779.5	0.0664	2.3184	2.1891	4.5993	25.8529
	se	25.8	0.0030	0.0269	0.0516	0.1064	0.7506
2	n	18	18	18	18	18	18
	mean	804.1	0.0745	2.3378	2.2180	4.7680	27.1544
	se	24.3	0.0031	0.0250	0.0493	0.1016	0.7173
20	n	19	18	19	19	19	19
	mean	749.1	0.0698	2.3159	2.2902	4.8594	27.1437
	se	23.7	0.0029	0.0239	0.0474	0.0977	0.6894
50	n	19	19	19	19	19	19
	mean	783.3	0.0695	2.3003	2.1562	4.7422	26.4786
	se	23.7	0.0028	0.0240	0.0475	0.0978	0.6899
200	n	15	15	15	15	15	15
	mean	700.5	0.1216**	2.3156	2.2243	5.3114**	29.7724**
	se	26.6	0.0056	0.0278	0.0551	0.1135	0.8010

Key: Significance level of comparison with control using William's test: * = p<0.05, ** = p<0.01
Significance level of comparison with control using Student's t-test: + = p<0.05, ++ = p<0.01
= Back transformed means and standard errors; data were logarithmically transformed prior to analysis.

Group mean organ weights adjusted for terminal body weight: females

Dose (mg/kg/day)		Terminal Bodyweight	Adrenal#	Brain	Heart	Kidney	Liver
0	n	17	17	17	17	17	17
	mean	448.7	0.0995	2.1270	1.5993	3.2253	16.5226
	se	15.8	0.0049	0.0216	0.0353	0.1151	0.5611
2	n	20	20	20	20	20	20
	mean	442.5	0.0885	2.1344	1.5406	3.0773	16.3145
	se	14.6	0.0040	0.0201	0.0328	0.1069	0.5212
20	n	20	20	20	20	20	20
	mean	479.4	0.0995	2.1418	1.5300	3.1151	16.5844
	se	14.6	0.0045	0.0199	0.0326	0.1062	0.5178
50	n	17	17	17	17	17	17
	mean	479.0	0.1128	2.1215	1.5532	3.2257	16.5120
	se	15.8	0.0055	0.0216	0.0353	0.1150	0.5609
200	n	13	13	13	13	13	13
	mean	474.2	0.1501**	2.1230	1.6057	3.8291**	19.5521**
	se	18.1	0.0084	0.0248	0.0402	0.1311	0.6392

Key: Significance level of comparison with control using William's test: * = p<0.05, ** = p<0.01
Significance level of comparison with control using Student's test: + = p<0.05, ++ = p<0.01
= Back transformed means and standard errors; data were logarithmically transformed prior

Gross and histopathologic findings included adrenal, pituitary and pulmonary changes. The sponsor does not separate the results into those who died ahead of scheduled euthanasia and those surviving to scheduled termination. Lungs and adrenals were analyzed from the LD and MD groups also.

Reviewer's summary of gross and histopathological changes

	Dose of ranolazine mg/kg/day				
(n=20 per group/sex)	0	2	20	50	200
Enlarged adrenal gland- f	0	0	1	2	7
Enlarged pituitary gland- f	2	2	4	5	10
Adrenal cortical vacuolation-f	4	2	2	3	19
m	13	15	6	19	20
Inhalation pneumonia f	0	0	1	3	8
M	2	0	0	5	15
Alveolar foam cell proliferation	2	5	2	2	7

Pk data showed that plasma levels increased with increasing dose. The increase in AUC values was greater than proportional from 2 to 20 mg/kg and from 20 to 50 mg/kg.

At the lowest dose, the AUC increased from 6 to 12 months, suggesting accumulation. There was slight increase in AUC from 6 to 12 months at 50 and 200 mg/kg, suggesting either accumulation or saturation of clearance.

Dose	C_{max} (ng/ml)		$AUC_{0-24\ h}$ (ng.h/ml)	
2 mg/kg/day	Male	Female	Male	Female
6 months	118 ± 32.4	334 ± 117	369	809
12 months	124 ± 31.8	500 ± 196	841	780

Dose	C_{max} (ng/ml)		$AUC_{0-24\ h}$ (ng.h/ml)	
20 mg/kg/day	Male	Female	Male	Female
6 months	3330*	5740 ± 700	7690	17600
12 months	2990*	6950 ± 410	7630	26800

Dose	C_{max} (ng/ml)		$AUC_{0-24\ h}$ (ng.h/ml)	
50 mg/kg/day	Male	Female	Male	Female
6 months	8900 ± 3260	8940 ± 191	28200	64500
12 months	5520 ± 3370	9020 ± 672	30100	71300

Dose	C_{max} (ng/ml)		$AUC_{0-24\ h}$ (ng.h/ml)	
200 mg/kg/day	Male	Female	Male	Female
6 months	17500 ± 11800	26800 ± 1840	132000	232000
12 months	27500*	29800*	202000	-

Values expressed in terms of ranolazine dihydrochloride.

C_{max} values were determined from the mean plasma level data and AUC values were calculated from these mean profiles.

C_{max} values are quoted as Mean ± SD for n=2 rats except for * where n=1.

The urinalysis data was presented in a format that was difficult to interpret. The mostly qualitative data was presented as single animal data. In the drug-treated males, starting with the first determination at 16 weeks, there appear to be increased incidence and severity of something found in the microscopy. This appears to be sperm, crystals and combinations of the two as well as some unspecified material in the "abno" column. There were findings of crystals for some of the HD males at 32 weeks. At the final determination, 48 weeks, there were microscopic findings of apparently dose-related frequency and severity for each of the groups of males.

Study title: 28-Day Repeated dose toxicity study of ranolazine free base containing RS94287 in Sprague-Dawley rats

Key study findings: RS94287, also known as Ran 2, is an impurity listed in the specifications for ranolazine, occurring at not more than 0.5% w/w (Item 4, vol 1, p. 56). The study was inadequate in design. This is essentially an interaction study between the impurity and the drug. The dose of drug used was a fraction of the human exposure and clinically irrelevant. There was no untreated group for comparison. Despite this, the data presented here showed a slight decrease in HCT in both sexes, accompanied in males by an increase in spleen weight and accompanied in females by an increase in MCV, suggestive of hemolysis and a regenerative response respectively. Organ weight data also showed a decreased weight of testes with increased concentration of impurity. In females, the MD and HD showed statistically significant increases in serum sodium and decreases in serum potassium. From the data available it cannot be concluded that this material is without biological effects.

Study no: 124-007
Volume #, and page #: vol 11, p.4
Conducting laboratory and location: Redfield Laboratories, Redfield Arizona
Date of study initiation: November 12, 1999
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-94287 (also known as Ran2) lot # 4007, ranolazine free base, lot E4-NE-002
Formulation/vehicle: control material was distilled water.

Methods: Ran 2 is an impurity listed in the specifications for ranolazine, occurring at not more than 0.5% w/w (Item 4, vol 1, p. 56). Ranolazine free base containing Ran2 was given by oral gavage each day for 28 days to Sprague-Dawley rats (Crl: CD® (SD)BR VAF, Charles River, N.C.). The study design is summarized in the table below.

Group designation *(percentage based on dosage level	Dosage level(mg/kg)	# of animals	
		Males	females

	Control (Ranolazine)	20	5	5
	Ranolazine + 1% Ran 2	20	5	5
	Ranolazine + 2.5% Ran 2	20	5	5
	Ranolazine + 5.0% Ran 2	20	5	5

It is not expressly stated what the dose of ranolazine is, but it is a reasonable assumption that 20 mg/kg is the dosage used. The sponsor notes in their table that the percentage of Ran 2 was based on the dosage level. Since the dosage of ranolazine was the same for all groups, this is not quite clear. It is also not clear to what the percentage of the impurity refers.

Animals were observed twice a day for morbidity and mortality. Body weights were recorded pre-test, approximately weekly, the day before necropsy and the day of necropsy. Ophthalmic exams were performed pre-test and prior to necropsy by a staff veterinarian. Blood samples were obtained prior to necropsy and analyzed for hematology and clinical chemistry parameters. Semi-quantitative urinalysis was performed on all animals at some time prior to necropsy. Study day 29, animals were euthanized and gross observations made. Organ weights were recorded for brain, liver, kidneys, testes, ovaries, adrenal glands, spleen and thymus. Histopathology was conducted for the control and 5% Ran2 group on the following tissues: adrenals, aorta, bone marrow, brain, cervix/vagina, epididymides, esophagus, eyes with optic nerve, femur, gross lesions, heart, cecum, colon, rectum, duodenum, ileum, jejunum, kidneys, liver, lungs, mesenteric and mandibular lymph nodes, mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostate gland, mandibular salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, uterus and urinary bladder.

Results: There were no treatment related signs. The male rats treated with Ran 2 showed less weight gain than the rats treated with ranolazine alone. This is summarized in the reviewer's table. There was no apparent effect on female weights.

Summary of weight changes in male rats.

	Ranolazine alone	Ranolazine + 1% Ran 2	Ranolazine + 2.5% Ran2	Ranolazine + 5% Ran 2
Absolute gain from baseline (g) Days 1-29	152 ± 31	138± 31	124± 28	134± 8
% of baseline	70%	65%	58%	63%

There was a slight decrease in HCT in both sexes. Mean cellular volume decreased slightly in males and increased significantly in females:

MCV(% cu microns pico grams) in female rats

ranolazine	56.8±1.51
Ranolazine + 1% Ran 2	58.5±1.44
Ranolazine + 2.5% Ran 2	61.1**±2.36 ($p<0.01$)
Ranolazine + 5% Ran 2	60.1*±1.60 ($p<0.05$)

There were no significant effects in the clinical chemistry results for the males. There were however, significant effects on Na and K in the female results, as shown in the reviewer's table below.

Significant Clinical Chemistry Results for Female Rats

group	Na mmol/l	K mmol/l
Ranolazine	144±0.4	7.2±1.07
Ranolazine + 1% ran 2	145±1.5	6.9±1.33
Ranolazine + 2.5% ran 2	148**±1.3	5.4*±0.63
Ranolazine + 5 % ran 2	147**±0.8	6.0±0.89

There were several organ weight effects in the male rats. This is summarized in the reviewer's table below.

Summary of organ weight effects in male rats

	ranolazine	Ranolazine 1% Ran2	Ranolazine 2.5% Ran2	Ranolazine 5% Ran2
Spleen as % body weight	0.188±0.0179	0.213±0.0162	0.229**±0.013	0.206±0.0206
Testes as % of body weight	0.902±0.0873	0.861±0.0379	0.865±0.0927	0.753±0.304
Liver as % of brain weight	626.7±82	661±132	590±72	581±41
Spleen as % of brain weight	34±3.6	39±1.4	40*±3.0	36±4.1
Testes as % brain weight	163±22	156±12	151±16	131±53

*significant at $p<0.05$, **$p<0.01$

The only histopathological finding possibly related to the organ weights was 1/5 HD males showed epididymal atrophy. In an appendix, it was noted that this was secondary to testicular degeneration.

There was no apparently drug-related effect in the urinalysis data.

Summary: The study was inadequately performed. This is essentially an interaction study between the impurity and the drug. However, the dose of drug used was a fraction of the human exposure and clinically irrelevant. Despite this, the data presented here showed a slight decrease in HCT in both sexes, accompanied in males by an increase in spleen weight and accompanied in females by an increase in MCV, suggestive of hemolysis and a regenerative response respectively. Organ weight data also showed a decreased weight of testes with increased concentration of impurity. In females, the MD and HD showed statistically significant increases in serum sodium and decreases in serum potassium. From the data available, it cannot be concluded that this material is without biological effects.

Study title: 28-Day repeated dose toxicity study of ranolazine free base containing RS88778 in Sprague-Dawley rats.

Key study findings: RS88778, also known as Di-Ran3-pip, is an impurity occurring at NMT 0.5% w/w (Item 4, vol 1, p.56). The hematology data was presented twice: once under the heading of hematology, once under the "Clinical Chemistry" section. The clinical chemistry data was found under the "Organ Weight" heading. The organ weight summary was not found although individual organ weights were found in Appendix 9(p.158). The urinalysis summary was found as an addendum on page 195. The sloppiness of the report makes one somewhat uncomfortable. The dose of ranolazine is clinically irrelevant. There was no untreated control group for comparison. The MD and HD males showed 11% and 16% less weight gain than the ranolazine controls. The study is poorly designed and inadequate; however, the difference in weight gain in the males makes it impossible to say that the compound is without biological effect.

Study no: 124-005
Volume #, and page #: vol 10, p.4
Conducting laboratory and location: Redfield Laboratories, Redfield, AZ
Date of study initiation: October 7, 1999
GLP compliance: statement was included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS88778 (Di-Ran3-pip) lot # EE-315-64, ranolazine free base lot # E4-NE-002
Formulation/vehicle: deionized water

Methods: Male and female Crl: CD® (SD) BR VAF /Plus out-bred albino rats (Charles River, MI) were given daily oral doses of ranolazine or ranolazine + Di-ran3-pip at 1%, 2.5% or 5% (percent of what we are not sure). The study design is shown in the table below.

Summary of study design

Group	Dose of ranolazine (mg/kg/)	# of animals	
		males	females
Ranolazine (control)	20	5	5
Ranolazine + 1% Di-ran3-pip	20	5	5
Ranolazine +2.5% Di-ran3-pip	20	5	5
Ranolazine +5 % Di-ran3-pip	20	5	5

Observations for mortality and moribundity were made twice daily. Body weights were recorded pretest, approximately weekly, on the day prior to necropsy and day of necropsy

An ophthalmological exam was conducted pre-test and prior to necropsy by a staff veterinarian. Blood samples were collected prior to necropsy and analyzed for standard hematological and clinical chemistry parameters. Semi-quantitative urinalysis was performed at some point prior to necropsy. Day 29, All animals were euthanized and gross observations made. Organ weights were recorded for brain, liver, kidneys, testes, ovaries, adrenal glands, spleen and thymus. Samples for histopathology were collected from adrenal glands, aorta, bone marrow, brain, cervix/vagina, epididymides, esophagus, eyes with optic nerve, femur, gross lesions, heart, cecum, colon, rectum,duodenum, ileum, jejunum, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, mammary gland, ovaries, pancreas, pituitary gland, prostate gland, mandibular salivary gland,

sciatic nerve, seminal vesicles, skeletal muscles, skin,spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, uterus and urinary bladder.

Results: There were no clinical signs referable to drug treatment. There was no apparent affect upon female weight but male weight was decreased with increasing dose of the impurity as summarized in the reviewer's table.

Summary of weight changes

	ranolazine	Ranolazine + 1% Di-ran3-pip	Ranolazine + 2.5%Di-ran3-pip	Ranolazine+ 5% Di-ran3-pip
Absolute change from baseline (g) Days 1-29	119.2± 44.7	128.6±29.9	95.8±43.6	90.4±21.0
% difference from baseline	48	53	37	32

There were no significant effects apparent in the hematology data for either sex. The clinical chemistry data was presented under the organ weight section, apparently in error as the hematology data was shown twice, once under the clinical chemistry heading and individual animal hematology data was presented in the organ weight section. The organ weight summary was not found. Individual animal data was presented in Appendix 9. Urinalysis data was found as an addendum to the report. There were no findings of significance in the data as presented.

TOXICOLOGY SUMMARY:

Ranolazine is the racemic mixture of the (+)R enantiomer (RS43285-198) and the (-)S enantiomer (RS43285-197). There was no presentation of a systematic, organized characterization and comparison of the relative toxicities of the racemate versus the enantiomers. It has been proposed to market the drug as a racemic mixture based upon the assumption that the enantiomers possess equal pharmacological and toxicological characteristics. The data to support that assumption was not found in the data presented. The drug is also highly metabolized. The pharmacology/toxicology characterization of the metabolites is incomplete.

Reports were presented for:

Acute oral and intravenous dosing studies in mice, rats and dogs
One month oral and iv dosing studies in rats and dogs
3 month oral study in mice
3, 6 and 12 month oral dosing studies in rats
3, 6 and 12 month oral dosing studies in dogs

Special toxicology studies included:

Acute adrenal function in rats
A subsequent modified acute adrenal function study
One month adrenal function in rats
In vitro studies of adrenal steroid release ± ACTH stimulation and ± steroidal precursors

Studies in mice

Oral EMLD study in mice: A single oral dose of 250 mg/kg caused severe clinical signs of subdued behavior, hunched stance, piloerection, hyperventilation and prostration in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing. A single oral dose of 50 mg/kg produced no signs.

Intravenous EMLD in mice: A single intravenous dose of 20 mg/kg produced no signs. A single intravenous dose of 30 mg/kg caused signs including hyperventilation, ataxia, piloerection, subdued behavior and prostration. Recovery was reported to occur within 1 hour of dosing.

Three-month oral dose ranging study in mice: Animals were dosed with 0, 5, 50, 100 or 200 mg/kg/day of ranolazine. The study was terminated at 8 days due to the deaths of animals at 50(3/10m), 100(1/10m, 3/10f) and 200(2/10m, 4/10 f) mg/kg. Clinical signs reported in this study-included sedation, hunched stance and prostration. The 100 and 200 mg/kg males gained on average 45% and 61% less than the control males.

Three-month oral dose ranging study in mice: mice received oral doses of 0, 5, 15, 25 or 35 mg/kg/day ranolazine. Final body weight was inconsistently affected and the results cannot be given any toxicological significance. There was a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated animals. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species. There were few findings of toxicological significance in this study.

Studies in rats

Oral EMLD study in rats: Five male and five female rats per group received single oral doses of either 250 or 500 mg/kg. The lower dose produced 40% mortality while the 500-mg/kg dose caused 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis. Survivors were euthanized after 14 days with no gross or histopathological observations made.

Intravenous EMLD study in rats: The rats received a single intravenous dose of 30 mg/kg. No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is greater than 30 mg/kg.

Comparative EMLD study in rats: Five male and five female rats per group were given single oral doses of 250 mg/kg or either the racemic mixture or one of the enantiomers. All animals showed signs of sedation, prostration, ataxia and dyspnea. The females receiving the racemic mixture had a later onset of signs (1.5 hours vs 12-38 minutes) compared to the animals receiving the enantiomers. The results were inconsistent with other studies that also found salivation, tremors and convulsions as well as earlier onset of signs with the racemic mixture.

One month intravenous toxicity study in rats: Twelve rats per sex per group were given daily intravenous doses of ranolazine of 0, 1, 5 or 25 mg/kg for 28 days. Immediate salivation,

sedation and convulsions followed iv administration of 25 mg/kg to both sexes of rats. At 25 mg/kg, 1/12 males and 1/12 females died. Increased liver weight (3%, 11% and 10% of control from LD to HD) and decreased uterine weight (-17%, -19% and –12% of control, from LD to HD respectively) were seen in drug-treated females. The uterine weight change was significant in the LD and MD groups at $p<0.05$. Increased spleen (4%, 13% and 13% of control from LD to HD respectively) and adrenal weights (4%, 18% and 13% from LD to HD) were reported for the drug-treated males. No NOEL was determined for the organ weight effects in either sex.

Three month oral toxicity data in rats: Twelve rats per sex per group were given once daily oral doses of ranolazine at 0, 5, 50, 250 and 500 mg/kg/day for 91 days. Unscheduled mortality was seen at 5 (1f), 250 (2m) and 500 (3m, 4f) mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg) and convulsions (≥250 mg/kg). The HD group also showed ptosis/sedation. Clinical chemistry findings in both sexes included increased serum sodium at the two highest doses at 8 and 12 weeks and increased alkaline phosphatase. Glucose was increased in the two highest dose groups of females. There were histopathological findings of adrenal vacuolation, centrilobular hepatocyte enlargement and pulmonary alveolar foam cell proliferation. The incidences are summarized in the table below.

Reviewer's summary of reported microscopic findings

finding	Males	Females
Centrilobular hepatocyte enlargement	0/12 ©, 1/12 HD	0/12 ©, 7/12 (HD)
Pulmonary alveolar foam cell proliferation	1/12©, 6/12 HD	0/12©, 2/12 (HD)
Adrenal cortical cell vacuolation	0/12 ©, 12/12 HD	0/12©, 7/12(HD)

*The sponsor stated in the text that vacuolated adrenal cortical cells were found in "a number of" 250 mg/kg (MD) males.

A complete assessment of the toxicological effects and independent interpretation of the study cannot be made from the material presented.

Six month oral toxicity study in rats with a one-month recovery period: Sprague-Dawley rats were assigned to groups with 30 males and 30 females in the control and HD groups. There were 20 males and 20 females in each of the LD and MD groups. At the end of the dosing period, all LD and MD and the first 20 rats in the control and HD groups were euthanized. The remaining 10 rats in the control and HD groups were given a 30 day drug-free recovery period. Doses used were 0, 5, 50 and 200 mg/kg.

Clinical signs were reported for doses ≥50 mg/kg/day and included the salivation, sedation, prostration, tachypnea or shallow breathing, hunched appearance, ataxia, piloerection and convulsions noted in other studies.Signs were usually observed within 1-2 hours after dosing and showed partial to complete resolution by 4-6 hours post-dose. Target cells reported in the hematology results may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes as has been seen before in this species. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were not susceptible to easy interpretation. The

adrenal and liver weights were increased in both sexes as summarized in the table below. It may be seen that liver and adrenal weight increases persisted into the recovery phase for the females. There were no apparent organ weight effects in the recovery males.

Reviewer's summary of organ weight data

Males: dose mg/kg/day				
	0	5	50	200
Adrenal	0.063±0.01	0.065±0.01	0.065±0.011	0.089**±0.016
Liver	16.23±2.96	15.42±2.16	16.08±2.11	17.66±2.71
Females				
Adrenal	0.074±0.011	0.071±0.012	0.083±0.019	0.108**±0.019
liver	9.48±1.09	9.08±0.95	9.99±1.22	11.04**±1.51
Female weights at end of recovery				
adrenal	0.067±0.017			0.082*±0.011
liver	8.92±0.63			9.64±1.23

*p<0.05, **p<0.01

The histopathology results were not entirely consistent with the previous study in that adrenal vacuolation was reported only for the males. The adrenal effects are summarized in the table below.

Reviewer's summary of adrenal gland findings

	Dose group (mg/kg/day)			
	0	5	50	200
Diffuse vacuolation of the zona fasiculata (males only)	0/20	6/20	9/20	18/18
Diffuse cytoplasmic foaminess of the zona fasiculata (females only)	0/20	0/20	0/20	17/20
Gross adrenal enlargement	1/20 MD f, 9/20 HD f 1/20 HD m			

One year oral toxicity study in rats : Male and female rats, 20/sex/group were orally gavaged once a day for 1 year with 0, 20, 50 or 200 mg/kg/day ranolazine. Signs were reported for doses ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half-closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male. The HD males gained on average 15% less body weight than the control group ($p<0.05$ by Student's test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups. Both sexes at the HD showed slight decreases in HB and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. Adrenal weight was increased in HD males and females at 50 and 200 mg/kg. Adrenal cortical vacuolation was increased in the HD females. Liver weight was increased in the HD groups of both sexes. Findings are summarized in the tables below.

Reviewer's summary of organ effects

	Dose group (mg/kg)				
N=20 per group per sex	0	2	20	50	200
Enlarged adrenal gland- females	0	0	1	2	7
Enlarged pituitary gland- females	2	2	4	5	10
Adrenal cortical vacuolation- females	4	2	2	3	19
Males	13	15	6	19	20
Inhalation pneumonia- females	0	0	1	3	8
males	2	0	0	5	15
Alveolar foam cell proliferation	2	5	2	2	7

Reviewer's summary of organ weight changes

Dose group	Adrenal weight		Liver weight	
Mg/kg/day	males	females	males	females
0	0.0066±0.003	0.095±0.005	25.85±0.75	16.52±0.56
2	0.0745±0.003	0.089±0.004	27.15±0.72	16.31±0.52
20	0.0698±0.003	0.099±0.005	27.14±0.69	16.38±0.52
50	0.0695±0.003	0.1128±0.006	26.48±0.69	16.51±0.66
200	0.1216**±0.006	0.1501**±0.008	29.77**± 0.80	19.55**± 0.70

The multiples of human therapeutic doses were calculated from 5 days of orally dosed 1000 mg of ranolazine, t.i.d. The human AUC at this dose was 33700 ng.hr/ml.

Summary of exposure relative to humans

Rat dose	AUC_{0-24} ng.hr/ml At 12 months		Multiple of human maximum dose	
	Males	Females	males	Females
2	841	780	0.013x	0.01x
20	7630	26800	0.11x	0.40x
50	30100	71300	0.45x	1.07x
200	202000	Not given		

The urinalysis data indicated a dose-related change in the samples from the males. Incidence and severity of sperm and crystals present in the urine was noted. From the data presented it is not known if the sperm were normal or abnormal in morphology.

Studies in dogs

Maximum tolerated intravenous dose study in dogs: Two pairs of 1 male and 1 female were given once daily intravenous injections on the following schedule:
Pair 1: 7 days at 10 mg/kg/day
7 days at 20 mg/kg/day

Male: 1 day at 40 mg/kg/day
Female: 21 days further at 20 mg/kg/day

Pair 2: 15 days at 20 mg/kg/day

Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received the dose and for approximately 15 minutes thereafter. At 20 mg/kg, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting either increased tolerance to the dose or induction of metabolism. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle was found on gross necropsy. While ECG tracings were generated, only heart rate data was provided. The dose of 20 mg/kg/day was tolerated for 21 days by the 1 female who received it.

Maximum tolerated oral (intubation) dose study in dogs: Two pairs of Beagles, one male and one female per pair were orally intubated once each day. Pair one received 50 mg/kg/day for one week followed by 100 mg/kg/day for a week. Both dogs then received an additional single oral dose of 150 mg/kg/day. Pair two was dosed at 100 mg/kg for 9 days. This was discontinued due to marked clinical signs which included combinations of sedation, ataxia, muscle tremors, vomiting, salivation and on one occasion in the female, prostration. On day 9, both dogs were found prostrate and trembling 2 hours after dosing. The male appeared unaware of his surroundings. Partial recovery was seen within 2-4 hours. After a 7 week recovery period, dosing was resumed at 80 mg/kg/day for 2 weeks.

Single doses of 50 and 80 mg/kg caused vomiting within 45 minutes of dosing. Repeated doses of 80 mg/kg caused sedation, muscle tremors, mild ataxia, salivation, vomiting and prostration within 1 hour of dosing. Recovery took 3-5 hours.

A single dose of 100 mg/kg in naïve animals produced no signs. Continued dosing at this level produced the signs already noted above. When the 100 mg/kg dose followed a week of dosing at 50 mg/kg, the signs included sedation, muscle tremors, mild ataxia and staining of the mouth (salivation?) in the male and no reported signs in the female. The female vomited on most occasions within 60 minutes of dosing and showed sedation on several occasions 1-2 hours after dosing. Recovery was within 6-7 hours.

One month intravenous toxicity study in dogs: Three male and three female beagles per group were given daily intravenous injection of 0, 1,5 or 20 mg/kg/day of ranolazine.

There was no unscheduled mortality. Signs were reported predominantly for the HD group and included “subdued behavior” almost daily for all HD animals. This began immediately after dosing and lasted from 5 minutes to 1 hour. There were also reports of vomiting, trembling and hind limb ataxia. One female showed ataxia almost continuously during weeks 3 and 4. Conjunctival congestion was reported for a HD male and a HD female. No incidence tables were given. Average body weight was dose-dependently decreased in the males but apparently unaffected in females despite sporadically decreased food consumption and significantly decreased food consumption in the last week in LD and MD females.

Although ECG tracings were reportedly generated, only single animal heart rate data was presented. The study is underpowered to find patterns, although there is a suggestion of a decrease in heart rate in treated animals at 5 minutes and 1 hour after dosing.

A brief pathological report showed only a mention of meningo-encephalitis in one HD female. The sponsor suggests that this was of viral origin. This raises concerns as to the standards of care used in the animal facility if viral meningo-encephalitis (canine distemper against which dogs are routinely vaccinated) found entry.

Oral investigative tolerance study in Beagle dogs with ranolazine administered three times daily: Two male and 2 female dogs were given escalating doses of ranolazine three times a day on the following schedule:

Days 1-7: 25 mg/kg tid ranolazine
Days 8-14 40 mg/kg tid
Days 15-21 50 mg/kg tid
Days 22-35 60 mg/kg tid

One male was euthanized day 29 some 24 hours after his last dose due to marked clinical signs of subdued behavior, thrashing legs, trembling and tachypnea. Plasma drug levels were determined but not reported. Signs reported at 25 and 40 mg/kg tid included green feces and occasional vomiting. Salivation was mild at 50 mg/kg/tid and more pronounced at 60 mg/kg. At 60 mg/kg, peripheral vasodilation as evidenced by the ears was also pronounced as were trembling and subdued behavior. Day 35, one female was observed in lateral recumbency, legs flaying, barking, trembling and salivating. This lasted some 4 minutes with recovery ~3 hours later.

Of the ECG data, only heart rates were provided. The hematology and clinical chemistry data were for 1 dog/sex. This underpowered study provides little information. The sponsor proposes hypotension as a cause of the clinical signs but presents no data to support this. It is not clear that hypotension alone would cause a recumbent dog to thrash and bark.

Four week investigative study in dogs:
This study was originally to evaluate local gastrointestinal effects of a sustained release tablet. Two male Beagles were assigned to the control group and 4 to the treatment group. The animals were dosed once a day, approximating the target dose of 68.2 mg/kg/day. The sponsor states that this is equivalent to the 80 mg/kg/day of the dihydrochloride which was used in a 3 month oral study in dogs.

This single dose study with suboptimal reporting had few findings of toxicological significance.

Three month oral toxicity study in dogs: Clinical signs were noted for doses ≥25 mg/kg, and included salivation, vomiting, ptosis, glazed eyes, conjunctival congestion, sedation, ataxia, trembling and convulsions. “Subdued behavior” was especially apparent in the first month. Significant changes in hematology, clinical chemistry and urinalysis are not apparent. Absolute and normalized testicular and adrenal weight for the treated male dogs was increased over

control but not in a dose-dependent fashion. Uterine weight of the drug-treated females was decreased compared to the control group. The presentation of pathology findings was confusing and raised questions as to the consistency of observations made. Although ophthalmic exams were conducted there was no statement from an ophthalmologist. Although ECG tracings were obtained, only single animal heart rate data was presented. In both sexes of drug treated animals heart rate were decreased at the 1 hour post-dose observation time in week 1.

Organ weight data was presented for individual animals. In an underpowered study such as this one would not expect to be able to discern differences between groups. The reviewer calculated means for absolute and normalized organ weight from the data presented. This is summarized in the table below. The absolute and normalized weight of testes in the drug-treated groups was more than that of the control group but no pattern was discernible. Absolute and normalized adrenal weight was also increased

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (testes)	13.12	14.06	15.2	13.06	15.66
Normalized (testes)	0.95	1.13	1.20	1.02	1.088
Absolute (adrenal)	0.74	0.72	0.835	0.85	0.87
Normalized (adrenal)	0.054	0.058	0.066	0.067	0.060

Uterine weight was also decreased in all drug-treated groups. Adrenal weights in the females were not discernibly affected.

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (uterine)	8.05	6.86	3.41	4.78	2.89* 4.03**
Normalized (uterine)	0.75	0.59	0.29	0.40	0.24* 0.34**

* the sponsor had a footnote for one individual measurement "left horn only". ** the asterisked animal was omitted from the calculation

Decreased uterine weight was also seen in the one month intravenous dose study in rats.

Six month oral toxicity study in dogs: Dogs were assigned to 4 treatment groups with 4 males and 4 females per group. The dogs were dosed once a day for 26 weeks with 0, 5, 25 or 60 mg/kg/day. Signs were primarily reported for doses ≥ 25 mg/kg and included mydriasis with loss of pupillary light reflexes, glazed eyes, ptosis and other signs of sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups. Rouleaux were reported for the MD and HD males and 2 HD females.

Adrenal weights were slightly increased in the HD males while testicular weight was decreased in the HD group. Pituitary weight was increased in females in a dose-related manner.

Reviewer's summary of organ weight changes: Percent change from the control group

Males				
	Dose group (mg/kg)			
	0	5	25	60
Adrenals	-	0	+6	+16
Testes	-	0	0	+23
Females				
Pituitary	-	+43	+39	+57

One year oral toxicity study in dogs: Five Beagles per sex per group were dosed once a day with 0, 10, 25 or 60 mg/kg/day**.** The signs in this study were significant in that salivation, sedation, trembling, convulsions, personality changes, ataxia and skin conditions (¾ HD males) were noted. The sponsor ascribes these to hypotension and/or cardiovascular collapse but presents no data to support this. The sponsor also stated that the skin condition was unrelated to drug treatment. The dermatologist's report stated that a drug-related effect could not be ruled out based on information available. The minimal information regarding the skin condition indicates an immune-mediated condition. A tissue distribution study in albino and pigmented rats showed preferential distribution to the retina and the skin of pigmented rats. Reports exploring this have not been found in this file. Does the apparent predilection for melanin binding have any bearing upon the skin pathology?

ECG tracings were reportedly obtained but only heart rates were presented.

Ophthalmic examinations were conducted by a "veterinary consultant". It was not made clear if the consultant was an ophthalmologist.

Urinalysis data was essentially qualitative. Acronyms were used without definition, making interpretation difficult.

Absolute and normalized uterine weight of the MD and HD females was decreased compared to the controls. Prostatitis was reported for 0/5(control), 2/5 (LD), 1/5(MD and 2/5(HD) males. Organ results are summarized below.

Reviewer's summary of organ weight changes: percent difference from control

Dose of ranolazine	Adrenal		prostate	testes	uterus
(mg/kg/day)	male	female			
10	+25	0	+52	-9	+4
25	+10	0	+9	-11	-39
60	+14	+21	+9	+5	-27

There was no difference in $AUC_{0\text{-}24}$ between 6 months and 12 months. Females showed greater plasma levels than males at the HD. In both sexes the increase in plasma level with increasing dose was greater than linear. The human equivalent doses are summarized in the table below.

Summary of multiples of human therapeutic exposure

Dog dose	$AUC_{0\text{-}24}$ ng.hr/ml	Multiple of human dose

(mg/kg)	male	female	male	female
10	3108±567	3423±2926	0.09x	0.10x
25	8203±3177	8873±3924	0.25x	0.27x
60	29520±12432	40409±12199	0.89x	1.21x

Special Toxicology Studies

The sponsor states that the "…in vitro and in vivo studies conducted to delineate the observation of adrenalcortical hypertrophy in rats showed ranolazine has no effects of potential toxicological relevance (vol 2, p.40)." The reviewer feels that the studies provide potentially useful pharmacological and toxicological information. From the Special Toxicology Studies (see below in section VIII), the individual study results may be summarized as follows:

RS 43285 RQT(3): Acute adrenal function study in rats

After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively. The acute increase in tissue steroids with decreased plasma ACTH and corticosterone is not consistent with stress but is suggestive of an acute drug effect.

RS 43285 RQT(2): Acute adrenal function study in rats

From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia), clinical chemistry or histopathology (effects upon lymphoid organs) to assess the relative contribution of stress. From the data presented it can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine.

RS 43285 RQT: One month adrenal function study in rats

There was little difference between the treatment groups in terms of basal plasma ACTH and corticosterone. Following a defined stress, plasma and adrenal corticosterone increased to a greater extent in all drug-treated animals. Only the HD group showed slight increases both in serum cholesterol and triglycerides and in adrenal weight.

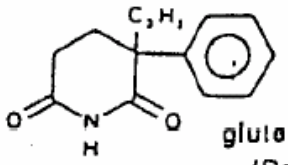
RS-43285: Investigative study in rat adrenal cells in-vitro

Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone. The study would be stronger for the inclusion of comparator compounds. However, the results as presented suggest either a physical or pharmacological interaction with ACTH or some non-competitive effect on the adrenal cells.

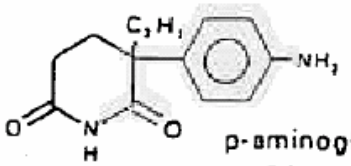
CL5566 In-vitro investigation of effects on adrenal tissue in rat, dog and man
Ranolazine inhibited both basal and ACTH-stimulated steroid (cortisol, coricosterone and aldosterone) secretion by rat, dog and human adrenocortical cells at concentrations $\geq 10^{-5}$ mol/l. Some effects were also seen at concentrations of 5×10^{-6}, 10^{-6} and 10^{-7} mol/l. Ranolazine also inhibited basal steroid release and release in the presence of precursors at essetially the same concentrations. It is not clear if the effect is from non-specific enzymatic interaction, cytotoxicity, or involvement in a step prior to production of 22-hydroxycholesterol such as delivery of cholesterol to CYP450 or the ACTH segment of the pathway.

Summary of Special Toxicology Studies

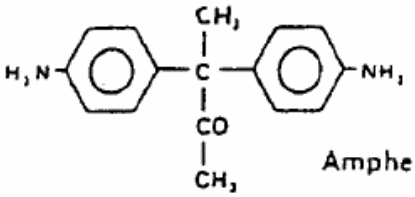
The studies indicate both acute and chronic effects of ranolazine on the adrenal gland. The basal circulating corticosterone levels were generally suppressed while tissue (adrenal) levels were increased. Although this effect is not consistent with a general stress response, hematology, clinical chemistry as well as histopathology of the lymphoid system are needed to support fully that conclusion. When a known, defined stress, usually physical restraint, was applied to the animals, an appropriate response of increased plasma corticosterone was seen in the drug-treated rats. The increase was however, less than the control group response in one study and greater than the control group in a second study. The studies in CL5566 showing that ranolazine caused a decrease in release of adrenal steroids in the basal state, after ACTH stimulation and in the presence of precursors were consistent with the other in vitro work. The laboratory that conducted that work also compared the ranolazine structure to that of known adrenocortical inhibitors such as glutethimide, p-aminoglutethimide, amphenone B, o,p-DDD(mitotane) and metyrapone. The sponsor's diagram is shown below.



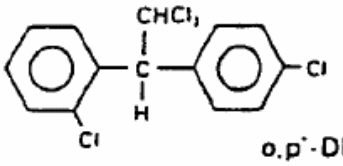
glutethimide
(Doriden)



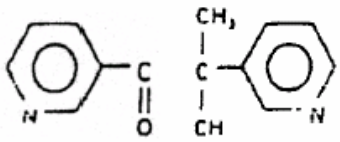
p-aminoglutethimide
(Elipten)



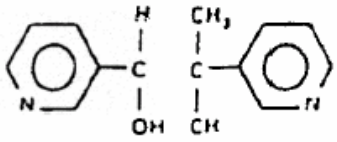
Amphenone B



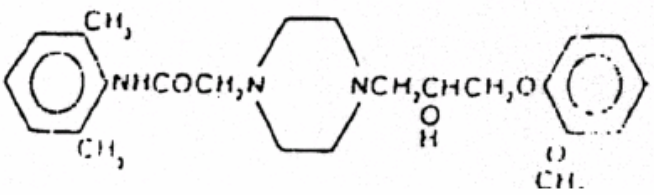
o,p'-DDD
(Mitotane)



Metyrapone
(SU4885)



Metyrapol
(reduced Metyrapone)



RS 13285

Initiated by the findings of adrenal lesions in two species in the general toxicology studies, the special studies indicate ranolazine's ability to influence adrenal gland function in the preclinical species. It is not clear whether this effect is direct or indirect. One possible indirect mechanism is opioid receptor binding. According to Goodman and Gilman (9th edition), opioid receptor binding can cause a decrease in ACTH secretion. Drolet et al., (Prog Neuro-Psychopharmacol &Biol Psychiat 2001, vol 25, pp729-741) noted that opioids can diminish stress-induced neuroendocrine and autonomic responses and may stimulate these effector systems in the non-stressed state. When the adrenal effects are taken into consideration with receptor binding studies, the neurological signs shown by the animals and the safety pharmacology studies, the combined data suggests a possibility that either the parent drug or a metabolite may be contributing opioid receptor binding to the pre-clinical picture. It is also possible that the adrenal effects are indirect support for the sponsor's proposed mechanism of pharmacologic action.That is, rate limiting components of adrenal steroid biosynthesis are mobilization and delivery of cholesterol to the inner mitochondrial matrix. Somehow ACTH stimulates this translocation by methods that are incompletely described. The rate limiting conversion in steroidal synthesis is the conversion of cholesterol to pregnenolone, another mitochondrial event (Goodman and Gilman 9th ed). If ranolazine does in fact alter the mitochondrial fatty acid oxidation process, are these events somehow secondarily or indirectly changed?

Overall Summary

There are several points of concern common to all the toxicology studies.

1. There is no margin of safety between the plasma levels causing adverse effects in the non-clinical species and the therapeutic plasma levels in humans. Wherever possible, the reviewer calculated exposure relative to humans using the ratio of AUC. The human AUC value should be multiplied by 2 to reflect the exposure that occurs with the proposed twice a day dosing.
2. There is evidence from the distribution studies conducted in pigmented rats that indicates melanin binding in the retinal pigmented epithelium and pigmented skin.
   Skin problems were reported in one of the dog studies. The dermatologist's report stated that a drug effect could not be ruled out.
   No ophthalmologist's report was located in any of the toxicology reports. There is no evidence that a qualified veterinary ophthalmologist has evaluated the eyes of the animals used in the toxicology studies. Of particular interest are the dogs, the only pigmented animals used.
3. Although it was stated in a number of studies that ECG tracings were obtained, the only data presented was single animal heart rate data.

4. The reproductive and developmental toxicology studies indicated effects on fertility. This was supported in several studies: a dose-related increase in pituitary size in female rats (1 year oral study) and a decrease in uterine weight was noted in a 1 month iv dosing rat study; a dose-related decrease in the absolute and normalized uterine weight in female dogs (3 month study). A dose-related decrease in absolute and normalized uterine weight was also seen in the 1 year oral toxicity study in dogs. In males, an increase in absolute and normalized testicular weight was reported in the 3 month dog study and the 6 month dog study. In the 1 year study, the weight of

the prostate was increased at all doses while testicular weight was decreased at LD and MD and increased at HD. The urinalysis data in the one year rat study indicated a dose-related change in the presence of sperm and crystals. There is insufficient information to evaluate the relevance of this finding to the question of fertility.

5. In both rats and dogs, adrenal weight was increased. Where pathology was reported, histopathological findings included diffuse vacuolation (males only), diffuse cytoplasmic foaminess of the zona fasiculata (females only). The mechanism of the adrenal changes is not clear and the relevance to humans is unknown at this time.
6. Neurologic signs were reported for essentially every study. Signs included sedation, ataxia, ptosis, salivation, dyspnea, tachypnea, piloerection, hunched appearance and convulsions. Loss of pupillary light reflex was noted in 2 dogs studies. The sponsor attributes these signs to hypotension but presents no data to support this. The sponsor does not use the word "sedation" to describe the animals' "subdued behavior and ptosis." The neurological evaluation of the safety pharmacology clearly indicated CNS effects of ranolazine, including sedation. Why is the word not used in the toxicology studies?

Toxicology conclusions: Preclinically, ranolazine has neurologic, cardiovascular and reproductive toxicities. The contribution of the metabolites to these adverse effects is unknown. The apparent melanin binding of drug and long elimination half-life raises a question as to the long-term effect upon the pigmented structures of the eye and pigmented skin. If the mechanism of action of ranolazine is a primary down-regulation of mitochondrial fatty acid oxidation, what effect will accumulation of drug have upon the mitochondria-rich retinal pigment epithelium? The study reports presented are suboptimal and leave questions unanswered as to the preclinical characterization of ranolazine.

Histopathology Inventory for NDA

Study	SS/028/85	SS/012/85	SS/047/87	AT6971	AT3280	AT3281	AT4050	AT6544	AT6543
Species	dog	Rat	rat	Dog	Rat		Dog	Rat	dog
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*
Aorta	x	X	X	X	x	x	x	X	X
Bone Marrow smear	x		X		x	x	x	X	X
Bone (femur)		X	X		x				
Brain	X*	X*	X	X*	X*	X*	X*	X*	X*
Cecum									X
Cervix	x	X	X	x	x	x	x	X	
Colon	X	X	X	x	x	x	x	X	X
Duodenum	X	X	X	x	x	x	x	X	X
Epididymis	X	X	X	x	x	x	x	X	
Esophagus	X	X	X	x	x	x	x	X	X
Eye	X	X	X	x	x	x	x	X	X
Fallopian tube									
Gall bladder	X			x		x	x		X
Gross lesions	X	X	X	x	x	x	x	X	X
Harderian gland			X					X	
Heart	X*	X*	X*	X*	x	X*	X*	X*	X*
Ileum	X	X	X	x	x	x	x	X	X
Injection site					x	x			
Jejunum	X	X	X		x	x	x	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland				x			x		X
Larynx									
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	x	x	x	x	X	X
Lymph nodes, cervical									
mph nodes mandibular			X		x			X	
mph nodes, mesenteric	X	X	X	x	x	x	x	X	X
Mammary Gland	X	X	x	x	x	x	x	X	X
Nasal cavity									
Optic nerves	X			x	x	x	x		
Ovaries	X*	X*	X	x	x	X*	X*	X*	
Pancreas	X	X	X	x	x	x	x	X	X
Parathyroid			X	x				X	x
Peripheral nerve									
Pharynx									
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*	X*	X*	X*	X*
Rectum									X
Salivary gland	X	X	X	x	x	x	x		X
Sciatic nerve	X	X	X	x	x	x	x	X	X
Seminal vesicles									
Skeletal muscle	X	X	X	x	x	x	x	X	X
Skin	X	X	X	x	x	x	x	X	X
Spinal cord	X		X	x	x	x	x	X	X
Spleen	X*	X*	X*	X*	x	x	X*	X*	X*

Sternum	X				x	x			
Stomach	X	X	X	x	x	x	x		X
Testes	X*	X*	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X	X	X*	X*	X*	X*	X*	X*
Thyroid	X*	X	X*	X*	X*	X*	X*	X*	X*
Tongue			X				x	X	
Trachea	X	X	X	x	x	x	x	X	x
Urinary bladder	X	X	X	x	x	x	x		X
Uterus	X*	X*	X*	X*	X*	X*	X*	X*	
Vagina	X	X	x	x			x		
Zymbal gland									
Standard List									

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

Study title: Bacterial reverse mutation assay for [redacted]

Key findings: [redacted] is an impurity present in the drug product at NM[redacted] w/w. This study is inconclusive. There was a disagreement in the interpretation of results between a technician and the study director that the study director chose to resolve by "reviewing selected plates that could be recovered following disposal of the plates " and doing replicate plating. It would be preferable to repeat the study de novo.

Study no: AA60LA.503.BTL
Volume #, and page #: Vol 14, p. 207
Conducting laboratory and location: BioReliance, Rockville, MD
Date of study initiation: June 18, 2002
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SAR-85-118, >98%

Methods: [redacted] was tested in the bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli WP2*uvr*A ±S9 activation. In the initial assay, the maximum concentration tested was 5000 µg per plate. Concentrations from 0.05 to 1.5 mg/ml were soluble and clear. Concentrations from 4-100 mg/ml were cloudy. Undissolved particles were present from 1.5 – 100 mg/ml. Concentrations tested were 2.5, 7.5,25, 75, 200, 600, 1800 and 5000 µg per plate. Precipitate was observed at concentrations ≥600 µg per plate. The concentrations tested in the confirmatory assay were 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg per plate. Precipitate was again observed at concentrations ≥ 600 µg per plate. The vehicle used was DMSO. Appropriate positive controls were used. Criteria for a positive response was set to include a "...dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean

vehicle control value. Datasets for the remaining tester strains were required to have a 2-fold increase in revertants.

Results: The study director apparently did not agree with the results generated by the technician conducting the study and so retrieved some plates from the biohazard waste and re-read them. To confirm the point, replicate plates were made of the plates retrieved from the garbage. The details are provided in the sponsor's paragraph below.

In Experiment B2 (Confirmatory Mutagenicity Assay), increases in the apparent revertant counts, consistent with the criteria for positive responses, were observed with tester strains TA100 and TA1537 in the absence of S9 activation and with TA100 and TA1535 in the presence of S9 activation. Since these responses were drastically different than those observed in Experiment B1, the Study Director reviewed selected plates from Experiments B1 and B2 that could be recovered following disposal of the plates. His initial assessment based on observation of the recovered plates was that intermediate colonies were present in the plates from Experiment B2, especially at the upper dose levels. No intermediate colonies were observed in the plates from Experiment B1. Intermediate colonies are colonies that are smaller than normal revertants but larger than background lawn colonies. This is a phenomenon that is seen occasionally and quite often intermediate colonies are not true revertants. To confirm this hypothesis, selected, recovered plates from Experiment B2 were replicate-plated onto histidine-free medium. The results of these replicate-platings are presented in parentheses next to the corresponding original plate counts in Tables 13 to 16. The results show that the apparent increases in revertant counts observed with TA100 are not true revertants; this doesn't appear to

Bacterial Mutation Assay
Summary of Results

Table 32

Test Article Id : [redacted]
Study Number : AA60LA.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	11 ± 1	162 ± 5	32 ± 1	8 ± 1	11 ± 1
2.5	12 ± 2	172 ± 20	22 ± 4	7 ± 1	9 ± 2
7.5	9 ± 0	199 ± 2	26 ± 1	7 ± 2	8 ± 2
25	14 ± 1	194 ± 44	23 ± 3	9 ± 4	10 ± 1
75	10 ± 1	249 ± 68	22 ± 2	14 ± 5	11 ± 3
200	12 ± 2	370 ± 37	48 ± 19	16 ± 4	10 ± 2
600	9 ± 1	356 ± 98	71 ± 19	21 ± 10	9 ± 1
1800	10 ± 3	486 ± 66	74 ± 7	27 ± 3	10 ± 2
5000	13 ± 1	764 ± 135	82 ± 8	27 ± 4	6 ± 3
Positive	104 ± 20	629 ± 85	591 ± 81	936 ± 170	115 ± 16

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 1	163 ± 10	14 ± 2	6 ± 2	11 ± 3
2.5	23 ± 4	169 ± 22	15 ± 1	8 ± 5	15 ± 5
7.5	14 ± 1	150 ± 12	10 ± 1	8 ± 2	9 ± 2
25	20 ± 3	183 ± 9	15 ± 5	6 ± 2	10 ± 3
75	17 ± 2	187 ± 36	9 ± 3	5 ± 1	11 ± 2
200	25 ± 3	192 ± 48	18 ± 3	7 ± 2	9 ± 1
600	20 ± 1	235 ± 39	29 ± 16	6 ± 1	10 ± 2
1800	16 ± 2	250 ± 15	29 ± 1	6 ± 2	7 ± 1
5000	21 ± 2	440 ± 58	182 ± 10	12 ± 3	7 ± 2
Positive	294 ± 24	585 ± 53	151 ± 61	56 ± 7	1015 ± 140

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Bacterial Mutation Assay
Summary of Results

Table 33

Test Article Id : Di-Ran3-pip
Study Number : AA60LA.503.BTL Experiment No : B3

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 2	137 ± 9	14 ± 3	6 ± 2	12 ± 1
2.5	11 ± 1	163 ± 12	17 ± 4	6 ± 3	18 ± 3
7.5	11 ± 1	148 ± 10	18 ± 4	6 ± 3	12 ± 1
25	11 ± 2	166 ± 13	15 ± 1	8 ± 1	16 ± 3
75	15 ± 4	199 ± 34	12 ± 4	6 ± 3	11 ± 1
200	14 ± 3	250 ± 57	31 ± 7	10 ± 3	12 ± 3
600	11 ± 3	157 ± 19	32 ± 9	12 ± 3	14 ± 1
1800	13 ± 3	200 ± 33	29 ± 5	15 ± 3	12 ± 4
5000	13 ± 4	139 ± 28	22 ± 7	11 ± 3	10 ± 1
Positive	100 ± 4	631 ± 16	202 ± 8	555 ± 148	84 ± 6

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	21 ± 3	132 ± 5	14 ± 3	6 ± 3	12 ± 2
2.5	23 ± 3	152 ± 23	17 ± 7	9 ± 2	14 ± 2
7.5	24 ± 2	113 ± 11	17 ± 3	5 ± 2	11 ± 1
25	18 ± 2	157 ± 25	17 ± 3	4 ± 2	14 ± 1
75	13 ± 5	157 ± 20	12 ± 2	6 ± 2	14 ± 3
200	14 ± 4	147 ± 26	15 ± 5	7 ± 3	14 ± 3
600	20 ± 3	184 ± 35	17 ± 6	5 ± 2	11 ± 1
1800	15 ± 5	173 ± 48	23 ± 2	6 ± 1	16 ± 2
5000	19 ± 2	201 ± 11	18 ± 2	5 ± 1	12 ± 2
Positive	187 ± 47	667 ± 27	80 ± 13	80 ± 7	251 ± 64

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

One may ask several questions. Apparently, the technician who originally generated the results either did not feel that intermediate colonies were present or was unaware of the phenomenon. Was an inadequately trained technician conducting the study and if so how much reliance can be placed on the work? Or, was this a disagreement of interpretation? In either case, the study should have been repeated de novo rather retrieving discarded plates.
The study is inconclusive.

Study title: Mutagenicity evaluation of RS43285 batch #11 in the Ames Salmonella/Microsome plate test

Key findings: Under the conditions of the assay RS43285 did not cause an increase in revertants.

Study no: AM0203, Litton Bionetics Number 20988
Volume #, and page #: Vol 14, p. 275
Conducting laboratory and location: Litton Bionetics, Kensington, MD
Date of study initiation: April 10, 1984
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS43285, batch 11, purity 100%

Methods: Strains used were TA-1535, TA-1537, TA-1538, TA98 and TA-100. Doses used were determined from a preliminary assay, the results of which were not shown. Doses of RS43285 dissolved in DMSO were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) μg per plate. The highest dose was reported to cause 100% toxicity. The compound was tested ±S9 activation. The drug solvent was used as a negative control. Positive controls for -S9 studies were reported as sodium azide, 2-Nitrofluorene and 9-aminoacridine. The positive control for +S9 was 2-amino-anthracene. Criteria for a positive result was observation of a dose-response over three test concentrations and an increase in revertants equal to or greater than three times the solvent control value at the peak of the dose response. It was also required that strains derived from the same parental strain both show similar responses. The sponsor also stated that positive results not confirmed by repetition were not considered positive.

Results: Cytotoxicity was not shown. A summary of concentrations tested, appearance of background lawn and # of colonies per plate is presumably from the dose-ranging study The positive control did not work for TA-100 (-S9) and produced an insipid result with activation. These results were found again in a repeat study. In a third study using only TA98 and TA100, the positive control finally produced an acceptable response. The assay with the two strains was repeated and the results confirmed.

Conclusion: To be completely in accordance with contemporary standards either S. typhimurium strain TA102 or E. coli wp2*uvr*A ±pKM101 should have been included in the tester strains used. However, the study is reasonable. Under the conditions of the assay, RS43285 did not cause an increase in revertants.

Study title: Mutagenicity evaluation of RS-43285-193 in the Ames Salmonella/microsome and mitotic gene conversion assay with yeast strain D4 plate test

Key findings: Cytotoxicity results were not apparent. Under the conditions of the assay, no positive findings were apparent.

Study no: AM0219 Syntex #917-Y-84-43285-193-VO/MU/AM
Volume #, and page #:vol 14, p.298

Conducting laboratory and location: Litton Bionetics, Inc, Kensington, MD
Date of study initiation: October 26,1984
GLP compliance: a statement was included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity:RS-43285-193, 100%

Methods:
Salmonella strains used were TA-1535, TA-1537, TA-1538, TA-98 and TA-100.
Doses used were determined from a preliminary assay, the results of which were not shown. Doses of RS43285 dissolved in DMSO were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) μg per plate. The highest dose was reported to cause 100% toxicity. The compound was tested ±S9 activation. The drug solvent was used as a negative control. Positive controls for -S9 studies were reported as sodium azide, 2-Nitrofluorene and 9-aminoacridine. The positive control for +S9 was 2-amino-anthracene. Criteria for a positive result was observation of a dose-response over three test concentrations and an increase in revertants equal to or greater than three times the solvent control value at the peak of the dose response. It was also required that strains derived from the same parental strain both show similar responses. The sponsor also stated that positive results not confirmed by repetition were not considered positive.

Doses used were selected from a preliminary toxicity test performed on the D4 strain. The doses chosen were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) μg per plate. Samples were processed ±S9 activation. The positive control for –S9 was N-methyl-N-nitro,N-nitrosoguanidine and for +S9 was sterigmatocystin. The negative control was the vehicle of DMSO. Criteria for a positive result was observation of a positive dose response over three concentrations with the highest increase equal to twice the solvent control value. A positive result that was not confirmed by repetition was not considered significant.

Results and Summary: Cytotoxicity results were not apparent. Under the conditions of the assay, no positive findings were apparent.

Study title: Mutagenicity Evaluation of RS-43285-193 in the <u>in</u> <u>vivo</u> mouse micronucleus assay

Key findings: The presentation of the study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

Study no:AM0225 918-Y-84-43285-193-MU-MN
Volume #, and page #: vol 14, p. 326
Conducting laboratory and location: Litton Bionetics, Inc, Kensington, MD
Date of study initiation: January 21, 1985
GLP compliance: statement was included
QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: lot #12, purity 100%

Formulation/vehicle: water

Methods: Male and female ICR mice (Charles River, Portage, MI), 5/sex/group were used. Single oral doses of 0 (vehicle control of deionized water), 30, 100 and 300 mg/kg and a positive control of triethylmelamine were used. Animals were euthanized by either CO_2 or cervical dislocation at 24, 48 and 72 hours after dosing. Criteria for a positive response included statistically significant dose-related increase in micronucleated PCEs or the detection of a reproducible and statistically significant dose-response for at least one dose-level. The sponsor states that the final decision was based on "scientific judgement".

Results: Control data was shown only for the 24 hour time point. As presented, the test article did not cause an in crease in micronuclei.

Summary: The presentation of the study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

Study title: AM0304: Mutagenicity test on RS-43285-193 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. Amended final report.

Key findings: The study is inadequate in design and yet positive results reproduced with metabolic activation.

Study no: Hazelton Laboratories # 9737-0-437, Syntex protocol 939-Y-86-43285-193-MU-CHO
Volume #, and page #: vol 15, page 1
Conducting laboratory and location: Hazelton Laboratories, Kensington, MD
Date of study initiation: February 10, 1987
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-193, 100%

Formulation/vehicle: Test article was dissolved in McCoys 5a culture medium.

Methods: Duplicate cultures were used at each dose level for the test article. Single cultures were used for the negative control, solvent control and at each of two doses of the positive control. Chromosomal aberrations were analyzed from the four highest doses from which results could be obtained and from only one of the positive control doses. Positive controls were mitomycin c (MMC) for –S9 and cyclophosphamide (CP) for +S9.

For –S9, cells were exposed to the test article until 2.5 hours prior to harvest. The cells were treated with colcemid for that 2.5 hour period. +S9, there was a 2 hour incubation with drug followed by washing and reincubation with culture medium for the appropriate interval of time. Colcemid was added 2.5 hours before the termination of the cultures.

Concentrations used in the range-finding study ran from 161 ng/ml to 4.84 mg/ml. Without activation, 161 µg/ml and 484 µg/ml caused 10% and 40% decreases in monolayer confluence and dose-related decreases in visible mitotic cells. The range of 161 mg/ml to 4.84 mg/ml caused "complete toxicity". A concentration range of 45µg/ml through 600 µg/ml was tested in a 20 hour assay. Concentrations reported were 150, 300, 450 and 600 µg/ml. With metabolic activation, the concentrations of 1.61 mg/ml and 4.84 mg/ml caused toxicity with no monolayer remaining. At 484 µg/ml, no toxicity was reported. Ten (100 µg/ml – 600 µg/ml) and 20 (400 µg/ml – 1.6 mg/ml) hour harvests were used for the aberration assay. Concentrations reported were 192, 288, 384 and 576 µg/ml for the 10 hour assay and 400 and 800 µg/ml for the 20 hour assay. One hundred metaphases were analyzed.

One hundred cells from each duplicate culture at four dose levels of drug and from each of the negative and solvent control cultures were analyzed for aberrations. From one positive control culture 25 cells were scored for aberrations. Gaps were not recorded.

Criteria for a positive control included consideration of overall aberration frequency, percentages of cells with any or more than aberrations, positive dose response, and ultimately, scientific judgement.

Results: Cytotoxicity was not shown in the same table with the aberration data. An increase in # of aberrations per cell and % cells with aberrations was reported for 576µg/ml +S9, 10 hour incubation. This was not seen in the 400 or 800 µg/ml concentrations incubated for 20 hours.

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
(Results from Individual Cultures)

Assay No.: 3737 Trial No.: I Lab Code Cy: 2177 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT		CELLS SCORED	NUMBER AND TYPE OF ABERRATION																NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
			NOT COMPUTED			SIMPLE			COMPLEX							OTHER					
			TG	SG		TB	SB	DM	ID	TR	QR	CR	D	R	CI	PU	GT				
CONTROLS NEGATIVE:	McCoy's 5a	100	6			1	2												0.03	3.0	0.0
SOLVENT:	McCoy's 5a 10 µl/ml	100	7	2															0.00	0.0	0.0
POSITIVE:	Cyclophosphamide 50 µg/ml	25	1	2		5	4		4		3				1				0.68	44.0	16.0
TEST COMPOUND: RS-43285-193																					
192 µg/ml	A	100	5			1													0.01	1.0	0.0
	B	100	5			1				1				1					0.03	3.0	0.0
288 µg/ml	A	100	13			2				1			1						0.04	4.0	0.0
		100	12	3		1	1													1.0	1.0
384 µg/ml	A	100	6			1								1						1.0	1.0
		100	8																	0.0	0.0
576 µg/ml	A	100	3	6		6	10												0.16	15.0	
	B	100	2	5		4	4												0.08	7.0	1.0

A dose response was seen in a repeat +S9 study. This result was repeated.

TABLE 5A

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
Cells Fixed 10.0 Hours After Treatment

Assay No.: 3737 Trial No.: III Lab Code Cy: 3117 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION															NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
		NOT COMPUTED			SIMPLE			COMPLEX								OTHER				
		TG	SG		TB	SB	DM	ID	TR	QR	CR	D	R	CI		PU	GT			
CONTROLS NEGATIVE AND SOLVENT:	200	11	1									3						0.02	1.5	0.0
POSITIVE: Cyclophosphamide 50 µg/ml	25	2	1		2			2	1	3								0.32	24.0*	8.0*
TEST COMPOUND: RS-43285-193																				
598 µg/ml	200	4			1	1						1						0.03	1.5	0.0
698 µg/ml	200	11			2	4			1			1						0.04	2.5	1.0
798 µg/ml	200	24	1		8	14			1	2								0.13	11.0*	1.0

*Significantly greater than the pooled negative and solvent controls, p<0.01

Note: TB=chromatid break
SB= chromosome break

TABLE 5B CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
(Results from Individual Cultures)

Assay No.: 9373 Trial No.: III Lab Code Cy: 3117 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION																NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
		NOT COMPUTED			SIMPLE			COMPLEX							OTHER					
		TG	SG		TB	SB	DM	ID	TR	QR	CR	D	R	CI	PU	GT				
CONTROLS NEGATIVE: McCoy's 5a	100	5	1									2						0.02	2.0	0.0
SOLVENT: McCoy's 5a 10 µl/ml	100	6										1						0.01	1.0	0.0
POSITIVE: Cyclophosphamide 50 µg/ml	25	2	1		2			2	1	3								0.32	24.0	8.0
TEST COMPOUND: RS-43285-193																				
598 µg/ml A	100	2			1	1						1						0.03	3.0	0.0
598 µg/ml B	100	2																0.00	0.0	0.0
698 µg/ml A	100	5				1						1						0.02	2.0	0.0
698 µg/ml B	100	6			2	3			1									0.06	3.0	2.0
798 µg/ml A	100	14	1		4	6			1	2								0.13	11.0	1.0
798 µg/ml B	100	10			4	8												0.12	11.0	1.0

9737-0-437

Summary: For an acceptable protocol, at time of harvest, the highest concentration should produce a significant decrease in degree of cell confluency, mitotic index or cell count (all greater than 50%). The results reported here cannot be attributed to excessive cytotoxicity as cell culture confluence was reported as 100% of the control for the tested range of concentrations with activation and 63% at the highest concentration tested without activation. With and without activation, cells should be exposed to drug for 3-6 hours and sampled at a time equivalent to approximately 1.5 normal cell cycle lengths (~24 hours for CHO cells) after the beginning of treatment. In this study, cells were exposed for ~17 hours to drug (-S9) or for 2hours (+S9). Harvesting for the –S9 cells was ~2.5 hours later (total 20 hours, slightly less than 1 cell cycle). Harvesting for the +S9 cells was ~8 hours later (total of 10 hours) or 18 hours later (total of 20 hours). The study is inadequate in design and yet positive results reproduced with metabolic activation. The results should be considered positive.

Study title:AM0393 Escherichia coli WP2 *uvr*A reverse mutation preincubation assay

Key findings: Under the conditions of the assay, none of the tested concentrations produced an increase in revertants, either with or without metabolic activation.

Study no: 934-Y-91-43285-193-MU-EC, lab study number TA217.502041
Volume #, and page #: Volume 15, page 41
Conducting laboratory and location: Microbiological Associates, Inc, Rockville, MD
Date of study initiation: 11/26/91

GLP compliance: statement was included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot#E9-ML-002/01, 100%

Formulation/vehicle: DMSO

Methods: RS-43285-193 was tested using the E. coli tester strain WP2*uvr*A ±S9 metabolic activation. A dose range-finding study examined concentrations of 0.3, 1.0, 3.3, 10, 33, 100, 333, 1000, 3333 and 5000 µg per plate. Reduction in colonies >50% was reported for 3333 and 5000 µg/plate +S9. No toxicity was reported for any concentration –S9. The same concentrations were used in the definitive assay. The positive control with metabolic activation was 2-aminoanthracene and the control without activation was methylmethanesulfonate.

Criteria for a positive response included a dose-response effect and at least a 2-fold increase in the number of revertants per plate over the mean number of revertants per plate of the appropriate vehicle control.

Results: Under the conditions of the assay, none of the tested concentrations produced an increase in revertants, either with or without metabolic activation.

Study title:AM0394 CHO/HGPRT Mutation Assay

Key findings: Under the conditions of the assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

Study no: lab # TA217.332016, sponsor # 935-Y-91-43285-193-MU-HGPRT
Volume #, and page #: vol 15, p. 76
Conducting laboratory and location: Microbiological Associates, Inc. Rockville, MD
Date of study initiation: Nov. 22, 1991
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot #E9-ML-002/01, 100%

Formulation/vehicle: DMSO

Methods:
In the preliminary assay, cells were exposed to concentrations of 0.1, 0.3, 1,3,10, 30, 100, 300 and 1000 µg/ml for 5 hours. Cloning efficiency was ≥that of the vehicle control. In the definitive assay, the concentrations used were 200, 400, 600, 800 and 1000µg/ml±S9. Relative cloning efficiency was decreased only in the positive controls.
Positive controls were ethyl methanesulfonate (EMS) and benzo (a) pyrene (B (a) P).
Criteria for a positive result included a dose-dependent increase in mutant frequencies with at least two consecutive doses showing mutant frequencies which are elevated above 40 mutants per 10^6 clonable cells.

Results: Under the conditions of the assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

Study title 0434 Salmonella/mammalian-microsome preincubation mutagenicity assay (Ames test) and Escherichia coli WP2*uvr*A reverse mutation assay

Key findings: Cytotoxicity was reported for 3500 µg and 5000 µg per plate without S9 activation and at 5000µg per plate with S9 activation. The toxicity reported for the highest concentration was moderate to complete. None of the tester strains showed an increase in revertants following exposure to the test article.

Study no: sponsor's # 900-Y-94-43285-003-MU-AMEC
Volume #, and page #: vol 15, p. 108
Conducting laboratory and location: Microbiological Associates, Rockville, MD
Date of study initiation: 9/20/93
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-003, lot 30933-P-100,

Formulation/vehicle: DMSO

Methods: RS-43285-003 was tested in the *Salmonella* and *E. coli* mutagenicity assay using tester strains TA98, TA100, TA1535, TA1537, TA1538 and WP2*uvr*A ±S9 activation. The positive control for the plates –S9 were: 2-nitrofluorene, sodium azide,9-aminoacridine and methyl methanesulfonate. Positive controls for the plates +S9 was 2-aminoanthracene. Concentrations tested were 250, 500, 1000, 2000, 3500 and 5000 µg per plate.

Criteria for a positive assay were listed as 1) a minimum of three non-toxic dose levels required to evaluate the assay data (toxicity criteria also described) 2) at least a two-three - fold increase depending upon the tester strain in the number of revertants per plate over the mean revertants per plate of the appropriate control 3)accompanied by a dose-response.

Results: Cytotoxicity was reported for 3500 µg and 5000 µg per plate both ± S9 activation. The toxicity reported for the highest concentration was moderate to complete. None of the tester strains showed an increase in revertants following exposure to the test article.

Genetic toxicology summary: RS-43285 has been tested in several versions of the Ames assay including Salmonella strains, yeast D4 strains and E. coli WP2*uvr*A. There are several early versions of the Ames assay that were done according to GLP protocols but are not acceptable by contemporary standards due to insufficient number of tester strains used. The most recent *Salmonella sp*. and *E. coli* WP2*uvr*A Ames assays were acceptable by current standards and show no positive responses in the test system. The drug has also been tested in an *in vivo* mouse micronucleus assay, chromosomal aberration assay using CHO cells and a CHO/HGPRT mutation assay.

Under the conditions of the CHO/HGPRT mutation assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

The presentation of the mouse in vitro micronucleus study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

The CHO chromosomal aberration assay was not done according to contemporary standards yet positive results were obtained. For an acceptable protocol, at time of harvest, the highest concentration should produce a significant decrease in degree of cell confluency, mitotic index or cell count (all by greater than 50%). The results reported here cannot be attributed to excessive cytotoxicity as cell culture confluence was reported as 100% of the control for the tested range of concentrations. With and without activation, cells should be exposed to drug for 3-6 hours and sampled at a time equivalent to approximately 1.5 normal cell cycle lengths (~24 hours for CHO cells) after the beginning of treatment. In this study, cells were exposed for ~17 hours to drug (-S9) or for 2 hours (+S9). Harvesting for the –S9 cells was ~2.5 hours later (total 20 hours, slightly less than 1 cell cycle). Harvesting for the +S9 cells was ~8 hours later(total of 10 hours) or 18 hours later (total of 20 hours). Positive results reproduced with metabolic activation.

Genetic toxicology conclusions: There was no evidence of genotoxicity either in the Salmonella/E.coli WP2uvrA reverse mutation assay either with or without S9 activation or in the CHO/HGPRT assay. However, the in vitro mouse micronucleus assay was inadequate and equivocal. Positive results were found in the CHO chromosomal aberration assay. The genotoxicity of this drug is incompletely characterized.

Labeling recommendations: The genotoxic potential of this drug is incompletely known and cannot be discounted.

VI. CARCINOGENICITY:

Study Title: Ranolazine: Three month oral dose ranging study in mice.

Key study findings: The study was terminated after 8 days due to the deaths of animals at 50, 100 and 200 mg/kg. Clinical signs reported in this study included sedation, hunching and prostration.

Study number: AT6424
Volume #, and page #: vol 27, p. 280
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: February 17, 1987
GLP compliance: statement included
QA report: yes () no (x)
Drug, lot #, and % purity:

Methods: CD-1(ICR)BR mice, 10/sex/group, were given daily oral doses of ranolazine at 0,5,50, 100 and 200 mg/kg/day for 8 days. The original intention was to dose for 3 months. Body weights were recorded weekly and food consumption was estimated weekly. Post-mortem examinations were conducted on those who died. Surviving animals were euthanized and discarded without further examination at the termination of the study.

Results: Fourteen animals died in the first week of dosing. Eleven of these deaths were considered to be treatment related. The deaths are summarized in the reviewer's table below.

Reviewer's summary of unscheduled mortality

Dose of ranolazine (mg/kg/day	# of animals M/F	Deaths M/F	comments
0	10/10	1/0	Malintubation
5	10/10	0/0	
50	10/10	3/0	1 malintubation
100	10/10	1/3	1 male was malintubated
200	10/10	2/4	

Signs were reported for all dose groups.
At 5 mg/kg: 1 m subdued and hunched before dosing and for 2.5 hours after days 5-7
50 mg/kg: 1 m subdued with staining on head
100 mg/kg: subdued behavior
200 mg/kg: 4m/3f subdued day 1, 1m/2f ptosis, 2 more females had a rough coat. There were other instances of subdued behavior and prostration.

In the eight days of the study, the 100 and 200 mg/kg/day males gained on average 45% and 61% less than the control males. Body weights in the females showed no recognizable pattern. Food intake amongst all groups was apparently unaffected. Due to the high number of treatment related deaths it was decided to terminate the study prematurely.

Study title: Three month oral dose ranging study in mice

Key study findings: There is a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated males. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species. There are few findings of toxicological significance in this study.

Study number: AT5989 SS/030/90
Volume #, and page #: vol 23, p 4
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: May 1987
GLP compliance:
QA report: yes () no ()
Drug, lot #, and % purity:

CAC concurrence:

Study Type (2 yr bioassay, alternative model etc.): dose ranging

Formulation/vehicle: water
Drug stability/homogeneity:
Methods: Five treatment groups of 10 mice/sex/group were established with Crl:CD-1 (ICR)BR animals (Charles River). Mice received oral doses of 0, 5,15,25 or 35 mg/kg/day each day for 91-92 days. Body weight and food consumption were recorded weekly. There was daily observation for clinical signs. Blood samples were collected from all surviving mice from all groups under anesthesia after 12 weeks of dosing. Samples from the first 5 animals per group were used for clinical chemistry and samples from the last five per group were used for hematology. Mice were euthanized 24 hours after the last dose. Gross observations were recorded. Tissues weighed were: adrenals, heart, kidneys and liver. Tissues collected for histopath were adrenals, heart, kidneys, liver, lung, altered tissue.

Results:
The information regarding clinical signs and mortality is somewhat unclear. The most frequently observed sign was subdued behavior. The sponsor states that "In those animals that died and were found to have been **misdosed** most were subdued and later cold to the touch for up to three days before death." There was no further elaboration on the term "misdosed." Eight animals were reported to die from malintubation. Seven animals died either during or within a day of the terminal bleed (6 controls, 1 @5 mg/kg, 2 @15 mg/kg, 3 @ 25 mg/kg and 2 @ 35 mg/kg).

Weight was inconsistently affected in all treatment groups. There were no apparent differences in food consumption.

Percent difference in final weight from the untreated control group

	Dose mg/kg/day			
	5	15	25	35
Males	-10	-22	+10	+10
females	+7	-7	-12	-11

There is a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated males.
There were no significant differences or trends in the organ weight data.

Summary: There are few findings of toxicological significance in this study. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species.

The summary of the Carcinogenicity studies that was presented to the Exec CAC is attached as Appendix I.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: Oral Fertility and Reproduction Study in Rats with RS-43285-193

Key study findings: This study was marked by suboptimal design, methodology and reporting. However, fertility was decreased in both sexes. The decreased fertility in males was repeated twice. Decreased implantation index was noted in females. Developmental delays were seen in the offspring in all drug-treated groups. These delays included eye opening, negative geotaxis, vaginal opening and survival. Therefore, even without maternal toxicity, there were detrimental effects upon the offspring.

Study no.: AT4136 116-R-86-43285-PO-RMF
Volume #, and page #: Volume 19, page 175
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: September 19, 1986
GLP compliance: no statement was located in the study, however, pages are signed and there is a QA statement.
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity:100%
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods:

Four groups of 20 male and 40 female rats (Crl:CD® (SD)BR:VAF PLUS, Charles River, Portage, MI) were given once daily oral doses of 5, 40 or 300 mg ranolazine/kg. Doses were based on the 3 and 6 month rat toxicology studies. After males had been dosed for 80 days and females for 14 days, the sexes were cohabited. Dosing of both sexes continued during the cohabitation period and until weaning of the F1 pups. After weaning the F1 pups, half the control P1 males and all LD and MD P1 males were euthanized and necropsied. Because of an apparent reduction in fertility the remaining control males and the surviving HD males were allowed to survive and were given two additional mating trials with nondosed females. The two additional mating trials followed dosing for 156 days and again after a 32 day recovery period. In the main mating study, animals were cohabited in the ratio of 2 females to 1 male. Due to the deaths of a number of the HD animals, 6 mating groups were housed in a ratio of 1 male and three females. Cohabitation was continued until at least 30 P1 females in each group showed evidence of mating.

The first 12-15 P1 females in each group with evidence of mating were euthanized on GD13, 14 or 15 for midgestation evaluations. The remaining P1 females, with or without evidence of mating, were allowed to litter.

The second generation phase of the study was conducted without further drug treatment.

Randomly selected male and female F1 pups within each dose group were mated at time of sexual maturity. They were then designated P2. Fifteen mating units per treatment group consisted of 2 females and 1 male per unit. Observations were made for the pups of P2 animals (F2) until weaning at 21 days post-partum. The cohabitation period was listed as " continued until at least 18 P2 females in each group showed evidence of mating."

F1 pups were examined for post natal developmental landmarks on specific days. However, "If all pups in a litter did not show a positive response on the first day of observation, the pups were again observed for these characteristics at the next scheduled day of observation for general clinical condition. … Postweaning developmental tests were conducted only on F1 pups selected for the P2 generation. Each weanling was tested individually on the day indicated (±2 days). If the weanling did not show a positive response at the age indicated, the test was repeated approximately 7 days later or until all animal in the group showed maturation characteristics."

Results:

Seven out of 20 HD males died, from 0.5 – 3 hours after dosing, usually following signs of collapse, convulsions, inactivity and with pulmonary lesions suggestive of gavage errors (p.190). There were ""infrequent recordings" of decreased respiration and pallor. HD females showed signs of inactivity, salivation and collapse. In both sexes, the signs lasted for up to 1 hour. From week 5 to the end of the study, most HD males were reported to show consistent salivation. Day 3 of the study, a number of animals (males) in all groups began to show signs consistent with sialodacryoadenitis. This was confirmed via the sentinel animals. The sponsor reports that approximately 40% manifested this sign by the end of week 1 and approximately 90% by the end of week 6. Residual clinical signs were reported to be gone by week 17. The sponsor states that 4/20 HD females were reported to have died also, presumably of other causes (gavage errors).

In the first 5 weeks of the study the HD males gained on average 24% less than the control group. By week 32, the end of the study, HD males had overall gained 5% less weight than the vehicle controls. The sponsor reported that there were no significant effects on body weight of either sex (p.180). The LD group showed inconsistent patterns of weight gain, possibly due to the swelling associated with the sialodacryoadenitis.

The body weight history for the females is somewhat confusing. The table titled " Summary of P1 pregnant female body weight " starts at week 13 pregnant female weights and goes to week 19. Since the gestation period for the rat is considerably less than 6 weeks, the summary is not clear. The next table is titled "Summary of P1pregnant midgestation sacrifice female body weights." The post-mating day 1 numbers do not correspond to any of the weights in the "summary of pregnant weights" table. Were the numbers for the interim sacrifice animals kept separate from the other body weights? A footnote says that the midgestation sacrificed females were not included in the computations after study week 14.

Sponsor's summary of reproduction status (reviewer's percentages in parentheses)

	group

Dose received (mg/kg/day)	0	5	40	300
P1 males				
Number cohabited	20	20	20	15*
# with evidence of mating (%)	18(90)	19(95)	19(95)	15 (100)
# impregnating at least 1 female* **	**19(95)**	**20**	**20**	**12 (80)**
P1 females				
Number cohabited	40	40	40	36**
# with evidence of mating (%)	**30(75)**	**30(75)**	**31(77)**	**25(69)**
Dams sacrificed at midgestation				
# pregnant	**14(100)**	**14(100)**	**14(93)**	**8(67)**
# not pregnant (% of total)	**0 (0)**	**0 (0)**	**1(7)**	**4 (33%)**
Dams allowed to litter				
# littered	14	16	14	8
# not littered and not pregnant	**2(12.5)**	**0 (0)**	**2(12.5)**	**5 (38)**
Number without evidence of mating	10/40(25)	10/40(25)	9/40(23)	11/36(31)
Number littered	2	3	6	3
# not littered and not pregnant	8/40(20)	7/40	3/40	8/36(22)

*five males died prior to initial breeding
**four females died prior to breeding
***includes females with and without evidence of breeding

The two additional studies done due to the initial indications of decreased male fertility are summarized below:
Study 176-R-87 Male rats were given RS-43285 orally at 300 mg/kg/day for at least 133 days before mating to untreated females. There was a reduction in fertility: 100 % of control males impregnated females compared to 69% of HD males.

Study 195-R-87 Male rats were given RS-43285 orally at 300 mg/kg/day for at least 156 days, followed by a 32 day recovery period before mating to untreated females. There was a decrease in male fertility: 100% of control males impregnated females compared with only 69% of HD males.

The sponsor goes on to state that there were several specific males who contribute to these findings:
Study 116-R-86 3 males, #413, #415 and #419
Study 176-R-87 3 males, #415, #419 and #435
Study 195-R-87 4 males #407, #415, #419 and #435

#413 died prior to subsequent matings and no histopathology was provided. The other rats showed histologic evidence of atrophic changes of the testes and/or epididymides. The sponsor felt that these results were incidental. For rats # 407, 415, 419 and 435 to be the sole cause of the 31% decrease in fertility in the last two studies, there could have been no more than 13 animals in the HD group. Given that there were only 15 animals surviving the initial period of dosing, this is entirely possible. However, an effect found in 31% of the animals cannot be dismissed out of hand as unrelated to drug treatment. It must also be emphasized that even with a month of drug-free recovery and no significant weight differences, the decreased male fertility, seen three times in this series of studies did not disappear, reverse or mitigate. The sponsor states that in the 3-

month rat oral tox study there were no changes in the male sex organs after dosing at 500 mg/kg/day. The histopath for the 3 month study was incomplete with no incidence or summary tables. Histopathology results for the 6 month rat study were not susceptible to easy interpretation.

The summary of P1 gestation indices for the midgestation sacrifice group showed a dose-related decrease in percent pregnant and a 25% increase in % pregnant with resorptions. There was a non-significant decrease in litter size and implantations with drug-treatment.

TABLE 4 (1 OF 2)
SUMMARY OF P1 GESTATION INDICES FOR MIDGESTATION SACRIFICE GROUP

GROUP	NO. DAMS	NO. PREG	% PREG	# WITH RESORP.	% PREG WITH RESORP.	# W/ALL FETUSES RESORB.
VEHICLE CONTROL	14	14	100.0	7	50.0	0
5 MG/KG/DAY RS43285-193	14	14	100.0	11	78.6	0
40 MG/KG/DAY RS43285-193	15	14	93.3	11	78.6	0
300 MG/KG/DAY RS43285-193	12	8	66.7	6	75.0	0

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
ITTER SIZE	VEHICLE CONTROL	14	14.2	2.29	0.567
	5 MG/KG/DAY RS43285-193	14	13.4	2.28	
	40 MG/KG/DAY RS43285-193	14	13.6	3.34	
	300 MG/KG/DAY RS43285-193	8	13.8	2.92	
TOTAL RESORPTIONS	VEHICLE CONTROL	14	1.0	1.11	0.295
	5 MG/KG/DAY RS43285-193	14	1.1	1.00	
	40 MG/KG/DAY RS43285-193	14	1.1	0.92	
	300 MG/KG/DAY RS43285-193	8	1.1	0.83	
IMPLANTATIONS	VEHICLE CONTROL	14	15.2	1.63	0.735
	5 MG/KG/DAY RS43285-193	14	14.5	2.31	
	40 MG/KG/DAY RS43285-193	14	14.6	3.27	
	300 MG/KG/DAY RS43285-193	8	14.9	3.27	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF PREGNANT FEMALES
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: IRDM JOB$0473 (11/05/87) JOB$0469 (11/05/87) SYW (11/18/87)

Summary of P1 Gestation Indices for Midgestation Sacrifice Group

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
CORPORA LUTEA	VEHICLE CONTROL	14	13.8	2.33	0.692
	5 MG/KG/DAY RS43285-193	14	13.7	2.16	
	40 MG/KG/DAY RS43285-193	14	13.1	2.16	
	300 MG/KG/DAY RS43285-193	8	14.8	1.28	
RESORPTION INDEX	VEHICLE CONTROL	14	8.9	7.88	0.389
	5 MG/KG/DAY RS43285-193	14	7.3	6.62	
	40 MG/KG/DAY RS43285-193	14	7.9	6.55	
	300 MG/KG/DAY RS43285-193	8	7.0	5.25	
IMPLANTATION INDEX	VEHICLE CONTROL	14	113.1	21.18	0.180
	5 MG/KG/DAY RS43285-193	14	107.3	18.79	
	40 MG/KG/DAY RS43285-193	14	112.9	25.51	
	300 MG/KG/DAY RS43285-193	8	100.3	19.75	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF PREGNANT FEMALES
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE
RESORPTION INDEX = (TOTAL RESORPTIONS/IMPLANTATIONS) X 100
IMPLANTATION INDEX = (IMPLANTATIONS/CORPORA LUTEA) X 100

SOURCE: IRDM JQB$0473 (11/05/87) JQB$0469 (11/05/87) SYW (11/18/87)

The summary indices for midgestational sacrifice group (dosed males mated with untreated females) shows decreased percentage pregnancies and fewer implantations.

TABLE 4A (2 OF 2)
SUMMARY OF GESTATIONAL INDICES FOR MIDGESTATION SACRIFICE GROUP
FOR 176-R-87-43285-193-PO-MR

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
RESORPTION INDEX	VEHICLE CONTROL	10	8.6	8.51	
	300 MG/KG/DAY RS43285-193	8	3.9	4.80	0.913
IMPLANTATION INDEX	VEHICLE CONTROL	10	117.5	17.57	
	300 MG/KG/DAY RS43285-193	8	90.6+	27.37	0.015

RESORPTION INDEX = (TOTAL RESORPTIONS/IMPLANTATIONS) X 100
IMPLANTATION INDEX = (IMPLANTATIONS/CORPORA LUTEA) X 100

* - MANN-WHITNEY TEST ONE SIDED P-VALUE ON ALL GROUPS

+ - INDICATES SIGNIFICANTLY LOWER THAN VEHICLE CONTROLS VIA THE MANN-WHITNEY TEST AT P = 0.05

NOTE: UNDOSED FEMALES MATED WITH DOSED MALES. N = NUMBER OF MALES WITH AT LEAST 1 FEMALE PREGNANT.

SOURCE: IRDM JQB$0255 (09/11/87) SYW$2599 (09/08/87)

When treated males were mated to treated females, the HD group had a pregnancy rate of 53% compared to 75% for the control group.

HD male rats treated for 133 days before mating with untreated females: 69% of the HD males impregnated females compared to 100% for untreated control males. Male rats treated for 156 days followed by a 32-day drug-free recovery period were mated to untreated females. 69% of the HD males impregnated females compared to 100% of the untreated control males.

The testicular and epididymal pathology results show that in the HD males who survived to necropsy, 2 out of 13 showed epididymal atrophy with 0-few spermatozoa present. One additional animal showed a decreased amount of spermatozoa. Incidence of these findings in all other groups was 0. Four/13 HD rats showed atrophy of the seminiferous tubules compared to 2/20 control rats. There was no quantitative examination of sperm numbers, motility or morphology apparent in the report. There was no mention of prostate gland or the seminal vesicles. No organ weights were listed.

The 4-day survival index was significantly decreased in the HD group and non-significantly decreased in the MD group: Control and LD groups: 100% survival, MD: 99% survival and HD: 95.59% survival ($p<0.05$ by Mann-Whitney). Survival index was also decreased day 7 (MD and HD), in all drug-treated groups days 14 and 21.

TABLE 5 (2 OF 2)
SUMMARY OF P1 GESTATION & F1 NEONATAL INDICES**

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
SURVIVAL INDEX DAY 7	VEHICLE CONTROL	16	100.00	0.000	0.016
	5 MG/KG/DAY RS43285-193	19	100.00@	0.000	
	40 MG/KG/DAY RS43285-193	20	99.38	2.795	
	300 MG/KG/DAY RS43285-193	11	96.59+	8.083	
SURVIVAL INDEX DAY 14	VEHICLE CONTROL	16	100.00	0.000	0.052
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	99.38	2.795	
	300 MG/KG/DAY RS43285-193	11	96.59	8.083	
SURVIVAL INDEX DAY 21	VEHICLE CONTROL	16	100.00	0.000	0.030
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	97.50	6.539	
	300 MG/KG/DAY RS43285-193	11	96.59+	8.083	
LACTATION INDEX	VEHICLE CONTROL	16	100.00	0.000	0.310
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	98.13	6.117	
	300 MG/KG/DAY RS43285-193	11	100.00	0.000	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
+ - INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE MANN-WHITNEY TEST AT P=0.05
@ - OBSERVATIONS FOR THIS GROUP EQUAL TO CONTROLS, NO COMPARISONS WERE MADE
** - FOR LITTERED FEMALES ONLY
DAY(I) SURVIVAL INDEX=(PUPS ALIVE DAY(I)/PUPS ALIVE DAY 1 AFTER CULLING) X 100
LACTATION INDEX=(PUPS ALIVE DAY 21/PUPS ALIVE DAY 4) X 100
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: IRDM JQB$0403 (10/27/87) JQB$0456 (11/04/87)

Mean pup weights in the HD group were decreased compared to control at all points of determination and reporting.

TABLE 6 (1 OF 2)
SUMMARY OF F1 MEAN PUP WEIGHTS (GRAMS) PER LITTER

MALES

AGE	GROUP	N	MEAN	STD DEV	P-LEVEL*
DAY 4	VEHICLE CONTROL	15	11..17	1.087	0.266
	5 MG/KG/DAY RS43285-193	18	11.23	0.930	
	40 MG/KG/DAY RS43285-193	20	11.20	1.066	
	300 MG/KG/DAY RS43285-193	11	10.57	0.937	
DAY 7	VEHICLE CONTROL	15	16.73	1.517	0.658
	5 MG/KG/DAY RS43285-193	19	16.75	1.943	
	40 MG/KG/DAY RS43285-193	20	16.98	1.542	
	300 MG/KG/DAY RS43285-193	11	16.08	1.826	
DAY 14	VEHICLE CONTROL	15	33.61	2.621	0.323
	5 MG/KG/DAY RS43285-193	18	33.93	1.852	
	40 MG/KG/DAY RS43285-193	20	33.56	2.490	
	300 MG/KG/DAY RS43285-193	11	32.39	2.946	
DAY 21	VEHICLE CONTROL	15	53.01	4.810	0.571
	5 MG/KG/DAY RS43285-193	18	55.10	3.870	
	40 MG/KG/DAY RS43285-193	20	55.23	5.401	
	300 MG/KG/DAY RS43285-193	11	51.21	4.288	

* - JONCKHEERE DOSE RESPONSE TWO SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF LITTERS WITH SURVIVING MALE PUPS
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: IRDM JQB$0404 (10/27/87)

Developmental parameters show that eye opening was delayed in all drug-treated litters compared to the control. Incisor eruption was delayed in the LD and HD groups. Negative geotaxis was also delayed in a dose-related manner.

TABLE 7 (1 OF 4)

SUMMARY OF F1 PHYSICAL AND BEHAVIORAL CHARACTERISTICS

CHARACTERISTIC (TEST NO: AGE)	GROUP	N	PROP$ OF LITTERS	P-* LEVEL	PROP@ PER LITTER MEAN	PROP@ PER LITTER STD DEV	P-** LEVEL
PINNA DETACHMENT (TEST 1:DAY 4)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
PINNA DETACHMENT (TEST 2:DAY 7)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
INCISOR ERUPTION (TEST 1:DAY 10)	VEHICLE CONTROL	16	0.13		0.46	0.356	
	5 MG/KG/DAY RS43285-193	18	0.06		0.33	0.357	
	40 MG/KG/DAY RS43285-193	20	0.10		0.43	0.343	
	300 MG/KG/DAY RS43285-193	11	0.09	0.434	0.19	0.351	0.077
INCISOR ERUPTION (TEST 2:DAY 14)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
FUR DEVELOPMENT (TEST 1:DAY 7)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
EYE OPENING (TEST 1:DAY 14)	VEHICLE CONTROL	16	0.25		0.57	0.345	
	5 MG/KG/DAY RS43285-193	18	0.06		0.38	0.322	
	40 MG/KG/DAY RS43285-193	20	0.10		0.46	0.340	
	300 MG/KG/DAY RS43285-193	11	0.09	0.122	0.24#	0.360	0.021

N = NUMBER OF LITTERS TESTED ON DESIGNATED DAY, - = NOT APPLICABLE
$ = PROPORTION OF LITTERS WITH ALL PUPS HAVING THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
@ = PROPORTION OF PUPS IN A LITTER WITH THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
* - ARMITAGE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
** - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
# - INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE ONE-SIDED MANN-WHITNEY TEST AT P=0.01

NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEOFRE COHABITATION UNTIL SACRIFICE
SOURCE: IRDM SYW$3173 (11/16/87), SYW$3133 (11/13/87), SYW$3162 (11/16/87), SYW$3187 (11/17/87), SYW$3183 (11/17/87), SYW$3206 (11/17/87)

TABLE 7 (2 OF 4)

SUMMARY OF F1 PHYSICAL AND BEHAVIORAL CHARACTERISTICS

CHARACTERISTIC (TEST NO: AGE)	GROUP	N	PROP$ OF LITTERS	P-* LEVEL	PROP@ PER LITTER MEAN	PROP@ PER LITTER STD DEV	P-** LEVEL
EYE OPENING (TEST 2:DAY 21)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00	-	1.00	0.000	
SURFACE RIGHTING REFLEX (TEST 1:DAY 7)	VEHICLE CONTROL	16	0.38		0.85	0.144	
	5 MG/KG/DAY RS43285-193	19	0.47		0.88	0.159	
	40 MG/KG/DAY RS43285-193	20	0.50		0.80	0.228	
	300 MG/KG/DAY RS43285-193	11	0.36	0.555	0.84	0.167	0.393
SURFACE RIGHTING REFLEX (TEST 2:DAY 10)	VEHICLE CONTROL	16	0.88		0.97	0.100	
	5 MG/KG/DAY RS43285-193	19	0.89		0.98	0.063	
	40 MG/KG/DAY RS43285-193	20	0.95		0.99	0.028	
	300 MG/KG/DAY RS43285-193	11	1.00	0.942¢	1.00	0.000	0.918
SURFACE RIGHTING REFLEX (TEST 3:DAY 14)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	0.95		0.99	0.029	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00	0.758¢	1.00	0.000	0.658
NEGATIVE GEOTAXIS (TEST 1:DAY 7)	VEHICLE CONTROL	16	0.25		0.62	0.375	
	5 MG/KG/DAY RS43285-193	19	0.21		0.58	0.347	
	40 MG/KG/DAY RS43285-193	20	0.10		0.54	0.297	
	300 MG/KG/DAY RS43285-193	11	0.00	0.024	0.41+	0.281	0.034
NEGATIVE GEOTAXIS (TEST 2:DAY 10)	VEHICLE CONTROL	16	0.75		0.97	0.061	
	5 MG/KG/DAY RS43285-193	18	0.83		0.95	0.130	
	40 MG/KG/DAY RS43285-193	20	0.75		0.96	0.085	
	300 MG/KG/DAY RS43285-193	11	0.73	0.388	0.96	0.066	0.384

N = NUMBER OF LITTERS TESTED ON DESIGNATED DAY, - = NOT APPLICABLE
$ = PROPORTION OF LITTERS WITH ALL PUPS HAVING THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
@ = PROPORTION OF PUPS IN A LITTER WITH THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
* - ARMITAGE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
** - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
¢ - EXACT DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
+ - INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE ONE-SIDED MANN-WHITNEY TEST AT P=0.05

NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEOFRE COHABITATION UNTIL SACRIFICE
SOURCE: IRDM SYW$3173 (11/16/87), SYW$3133 (11/13/87), SYW$3162 (11/16/87), SYW$3187 (11/17/87), SYW$3183 (11/17/87), SYW$3206 (11/17/87)

Vaginal opening was delayed in the MD and HD F1 females. While 100% of the control and LD offspring were reported to have opened between days 33-36, only 98% and 86% of the MD and HD rats respectively opened during that time. With a range of three days for the observation, some sensitivity is lost.

Fertility as evidenced by mating shows only 75% of the P1 untreated control females pregnant (30/40) and 53% of the HD females (19/36). In the P2 generation, only 67% of the untreated control P2 females (10/15) were reported pregnant. Of the drug-treated P2 offspring, 100% were reported pregnant. The untreated controls in each case show substantially lower mating and fertility values than the drug-treated animals of both generations.

Sponsor's Summary of P2 Reproductive Status (Reviewer's Percentages)

	Group			
Parental Dose mg/kg/day	0	5	40	300
P2 males				
Number cohabited	15	14*	15	15
Number with evidence of mating	14	13	15	14
Number impregnating at least 1 female	10	14	15	15
P2 Females				
Number cohabited	29*	30	30	30
# with evidence of mating	22(76)	24(80)	27(90)	18 (60)
# littered	15(52)	21(70)	25(50)	17(57)
# not littered and not pregnant	7	3	2	1
# without evidence of mating	7(24)	6	3	12(40)
#littered	1	4	0	2
# not littered	6(21)	2	3	10 (30)
#pregnant	0	0	0	1
#not pregnant	6	2	3	9

*one male and 1 female died prior to breeding.

Summary:
The study has several problems:

1. No organ weight data was provided.
2. No information was provided for the seminal vesicles or prostate gland
3. The cohabitation period (found in an Appendix) was listed as 5 days to 3 weeks if necessary. No detail was given in the report as to the time of cohabitation actually used for the different treatment groups or how many matings were necessary on average for the different groups.
4. The protocol did not specify and the report did not provide any information as to the cyclicity of the females.
5. The report did not specify methods of fixation or sectioning for what little pathology was done.
6. Sperm assessment including numbers, motility and morphology was not specified in the protocol nor mentioned in the report.
7. Any female that did not litter was euthanized and examined by necropsy approximately 1 week after the expected time of parturition for determination

of pregnancy status- not the most sensitive time for determination of fertility problems.

8. No table of data was found for those P1 females euthanized at the end of gestation rather than at midgestation.
9. Day 3 of the study, a number of males in all groups developed swelling in the ventrum of the neck. Approximately 40% of the males manifested this sign by the end of the first week and approximately 90% by the end of week 6. Sialodacryoadenitis (SDA) was diagnosed. Residual clinical signs were reported as absent by week 17 of the study. Why was the study continued at all given the incidence and duration of the disease outbreak? When an outbreak of SDA occurs, it typically goes through the entire colony with a single animal having signs for 7-10 days. There are two publications that report the virus causes irregular reproductive cycles in females (Macy et al. 1996. " Reproductive abnormalities associated with a coronavirus." Lab Anim Sci, vol 46, p 129-132; Utsumi 1991" Reproductive disorders in female rats infected with SDA". Exp Animal, vol 40, p 361-365.) as well as other reproductive and developmental problems.

Despite the suboptimal methodology, that there was a decrease in male fertility determined on the basis of dosing and breeding, a very unusual circumstance. This decrease was seen not once, but three times: in the initial P1 cohabitation/breeding(HD males impregnating females was 80% compared to the control group, 95%) the first extended dosing period of 133 days(HD males impregnated 69% of untreated females compared to the control males impregnating 100% of females) and in the group given the 32-day drug-free recovery period (69% of HD males impregnated untreated females compared to untreated males who impregnated 100% of untreated females). Because the rat has a large functional reserve, to see a decrease in fertility in such an insensitive parameter as breeding is a cause for concern. In the dose gap between 40 mg/kg and 300 mg/kg may well be a dose-response curve without parental clinical signs. The sponsor states that the decreases in male fertility in the HD group were due solely to 4 animals. Since the total group size for the surviving HD males was n=14, the 4 males in question represented 31% of the group. Although 2/20 control males were found to have atrophy of the seminiferous tubules, that group of males impregnated 100% of the females with whom they were cohabited.

It should also be noted that even without the data from the P1 end-of-gestation data, there was an apparent decrease in female fertility. The sponsor states in the summary that 53% of the HD P1 females were pregnant compared to the untreated controls where 75% were pregnant. The control fertility is low, but the HD females show a decrease that appears to go beyond the controls. At the midgestational euthanasia, there was 100% pregnancy in the control and LD groups, 93% pregnancy in the MD (with no maternal toxicity) and 67% pregnancy in the HD group. There was also an increase in percent pregnant with resorptions in all the drug-treated groups: 25-28% greater than the control group. Mean litter size was decreased in the drug-treated groups. Mean implantations were decreased in the midgestational data for the drug-treated groups, at the term euthanasia and in the extended study (176-R-87-43285-193-PO-MR, 133 days of dosing, a 27% decrease in implantation index compared to controls) in which untreated females were mated with HD males. There was no difference in implantation index when males were given a 32-day drug-free recovery period before mating. This is suggestive of a detrimental effect upon the quality of DNA in the sperm.

Developmental effects were also apparent. Pre-weaning mortality was significantly different from the control group in the HD pups on post-natal days 1,4,7 and 21. Eye opening and negative geotaxis were delayed in all drug-treated groups compared to the control pups. Vaginal opening was also delayed in MD and HD female pups. Given the insensitive nature of the method used to examine the developmental endpoints, the detection of any differences is impressive.

Study title: Oral Teratology Study in Rats with RS-43285-193

Key study findings: The study is suboptimal in design by contemporary standards. The HD produced such severe toxicity that there was an inadequate number of litters for evaluation if one uses the current guidelines. The sponsor argues that under the 1966 guidelines in place at the time of conduct, the study was acceptable. There was an increase in the incidence of reduced fetal ossification of the pelvic bones: 24% of the control litters vs 53% of the HD litters were affected. Misshapen sternebrae were also increased: control 52% of litters, LD 73% of litters, MD 69% of litters and HD 80% of litters. The sponsor appears to have analyzed the fetuses as the unit of comparison instead of the litter. If one looks at the above two parameters by the sponsors methods, the pelvic bone effect was control: 4%, LD 3%, MD 3%, HD 16%. Sternebrae: control 10%, LD 11%, MD 12% and HD 21%. Dysmorphic cranial ossification was increased in the LD and MD groups: control 52% of litters, LD 62%, MD 69% and high dose 33%. A HD that had not killed so many of the dams and had left more litters for evaluation may have shown completion of the dose response. No blood levels of drug are provided or referenced. From other studies (see the Carcinogenicity report) it is known that the LD and MD used produced fractional human equivalent doses. Based on a comparison of $AUC_{0\text{-}8}$, a 150 mg/kg dose in rats gave 0.7x the exposure of a human taking 30 mg/kg/day. Assuming a 70 kg person, this is approximately 2000 mg total dose or 1000 mg given bid. Given the inability to achieve a substantial multiple of human exposure in the animals, the conservative interpretation of this study would seem the most prudent. That is, that the excessive mortality at the HD prevented the clear delineation of the dose response curve and it should be interpreted that the drug causes teratogenic effects at low multiples of human exposure.

Study no.:AT3758 51-R-86-43285-193-PO-TT
Volume #, and page #: vol 17, p. 5
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: May 27, 1986
GLP compliance: no
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lots AT258SA2420, AT258SA320A, AT258/259SA224C aka AT258/259SA321A; 100%
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods: Sprague-Dawley rats (Crl:CD(SD)BR:VAF/Plus, from) with evidence of mating were randomly assigned to 4 groups of 27 females each. Rats were dosed with 0 (vehicle control), 5, 40 or 400 mg/kg/day RS-43285 by oral gavage from GD7 through GD16. After

3 days of once daily dosing the regimen was changed to twice daily dosing (half the dose given at each dosing) for all animals due to high mortality in the HD group. The rats were euthanized GD21. Females were observed daily with observations recorded on GD1, 7,14,16, 21, and days when a significant change was noted. Food intake was measured and recorded days 1,7,14 and 21. After euthanasia, the uterus was weighed and corpora lutea counted, live fetuses, early and late resorptions recorded. One-third of live fetuses were euthanized and processed for visceral examination. The remaining fetuses were processed for skeletal examination.

Results: 10 of 27 HD females died between study days 8-17 (4 deaths occurring after the switch to twice daily dosing). Other signs reported for this group included significantly lower food intake, listless and significantly lower gestational weight (28% lower than the control group), inactivity, labored respiration, slight to moderate ptosis, lack of mobility, unthrifty, cold, cyanotic and vocalizing post-dosing. Eight of 27 HD rats collapsed and had clonic convulsions after dosing (p.14). Four other HD rats collapsed post-dosing also. Mydriasis was also seen post-dosing as well as gasping, salivation and ocular and nasal discharge. There were no other reported signs of significance for the other drug-treated groups. Weight gain was comparable for the control, LD and MD groups.

There were no pregnant dams without fetuses in the control, LD or MD groups. There were however 9 pregnant dams without live fetuses in the HD group.

In the Summary of Gestational Indices (p.128), the "live litter size" is reported as:

group	Minimum live litter size	Maximum live litter size
Control	5.0	18.0
LD	6.0	19.0
MD	1.0	19.0
HD	1.0	19.0

Fetal weight was significantly ($p<0.005$) decreased in the HD group: 2.8 g vs 3.6 g for the control pups.

Number of corpora lutea showed a dose-related pattern even though dosing did not start until after the establishment of pregnancy:

Summary of corpora lutea(p.129)

group	Minimum corpora lutea	Maximum corpora lutea
Control	13.0	21.0
LD	13.0	20.0
MD	7.0	24.0
HD	4.0	21.0

Gestation survival index for all groups was shown as 100%. This is confusing in light of the 9 HD litters listed as "without live fetuses." The resorption index (total resorptions/implantations) was 11.3 for the HD group compared to 6.1 for the controls.

GROSS GROUP FETAL INCIDENCE TABLE FOR RATS GIVEN RS 43285-193

EXPERIMENT REGIMEN
(2 DAILY ORAL DOSE(S) DURING DAY 7 THRU DAY 16 OF GESTATION)
GROUP 0100 - VEHICLE CONTROL
GROUP 0200 - 5.000 MG/KG/DAY RS 43285-193
GROUP 0300 - 40.000 MG/KG/DAY RS 43285-193
GROUP 0400 - 400.000 MG/KG/DAY RS 43285-193
(FEMALES WERE NECROPSIED ON POST-MATING DAY 21)

GROUP	0100				0200				0300				0400			
PREGNANT DAMS IN GROUP	25				26				26				24			
PREGNANT DAMS WITHOUT LIVE FETUSES													9			
LIVE FETUSES IN GROUP	353				381				380				207			
EXTERNAL EXAMS IN GROUP	353				381				380				207			
SKELETAL EXAMS IN GROUP	231				250				253				134			
VISCERAL EXAMS IN GROUP	122				131				127				73			
	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %
NO. WITH ONE OR MORE CHANGES	25	100.0	181	51.3	26	100.0	187	49.1	26	100.0	183	48.2	15	100.0	127	61.4
EXTERNAL EXAMINATION																
NO ANOMALIES OBSERVED																
NO. OF FETUSES	25	100.0	351	99.4	26	100.0	379	99.5	25	96.2	379	99.7	15	100.0	207	100.0
AI :EY	1	4.0	1	.3												
DECREASED SIZE	1	4.0	1	.3	1	3.8	1	.3								
EDEMA									1	3.8	1	.3				
EYE/S																
ANOPHTHALMIA					1	3.8	1	.3								
PALLOR									1	3.8	1	.3				
RACHISCHISIS																
W/ EXENCEPHALY					1	3.8	1	.3								
SKELETAL EXAMINATION																
NO ANOMALIES OBSERVED																
NO. OF FETUSES	19	76.0	53	22.8	19	73.1	68	27.2	18	69.2	73	28.8	4	26.7	9	6.7
CRANIAL																
OSSIFICATION																
DYSMORPHIC	13	52.0	40	17.3	16	61.5	44	17.6	18	69.2	41	16.2	5	33.3	9	6.7
REDUCED	8	32.0	13	5.6	8	30.8	11	4.4	6	23.1	11	4.3	6	40.0	11	8.2
HYOID																
OSSIFICATION																
NON OSSIFIED	9	36.0	19	8.2	13	50.0	23	9.2	13	50.0	28	11.1	9	60.0	13	9.7
REDUCED	8	32.0	29	12.6	9	34.6	24	9.6	15	57.7	44	17.4	8	53.3	9	6.7

08/05/86 KEY: *-IN CASE OF LITTERING, THESE FIGURES INCLUDE PUPS

Significant effects on fetal weight were seen at the HD with median fetal weight 78% that of the control value. The incidence of reduced ossification of the pelvic bones was 16% in the HD group vs 4% in the controls using the sponsor's incorrect method of comparing fetuses rather than comparing litters. Misshapen sternebrae were increased across the drug-treated groups.

Summary

The study is inadequate. The HD produced such severe toxicity that there was an inadequate number of litters for evaluation. There is in the data a suggestion of several dose-related effects upon the developing fetus. A HD that produced only mild maternal toxicity might have helped to elucidate any fetal effects. The mortality that occurred has potentially obscured any teratological liability. No blood levels of drug are provided or referenced. From other studies (see the Carcinogenicity report) it is known that the LD and MD used produced fractional human equivalent doses.

Study title: Oral Teratology Study in Rabbits with RS-43285-193

Key study findings: Excessive maternal toxicity produced an inadequate number of litters for evaluation by contemporary standards. It may be seen in the existing data that in the absence of maternal toxicity, there was no NOEL for the observation of decreased implantation index.

Summary of implantation indices

Implantation index	87.7 (c)	73.8(LD)	64.3*(MD)	58.4**(HD)

(implantations/corpora lutea)				

significantly different from the controls by the Mann-Whitney test at *p=0.05 and **p=0.01

Reduced ossification of sternebrae was present in 54% of control litters and 77% of HD litters. There was a similar finding in the rat study. In this case, the effect is probably due to maternal toxicity. The LD and MD groups consumed the same amount of food as the control group but gained on average 38 and 66% more body weight than did the control animals.

Study no.: AT3802 92-B-86-43285-193-PO-TT
Volume #, and page #: Vol. 17, p. 158
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: September 29, 1986
GLP compliance: no
QA reports: yes () no ()
Drug, lot #, radiolabel, and % purity: lot numbers AT278SA331B,AT278SA248E, AT278SA244I, 100%
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods: 80 New Zealand White (NZW) rabbits (Nitabell Rabbitry of Hayward, CA) were randomly assigned to 4 groups of 20 females each and were then artificially inseminated (GD1). The groups were orally gavaged once daily with doses of 0 (vehicle control), 6, 45 or 150 mg/kg/day from GD7 through GD19. Dams were euthanized GD29. Body weights were recorded on gestation days 1, 8, 15, 22 and 29. Measurement of food intake began on the day following insemination and was recorded daily through GD29.

Results: Clinical signs were reported primarily for the HD animals and included increased respiration (10/20) and inactivity in 6/20, dyspnea, ataxia and collapse. Signs were reported to begin within 5-10 minutes of dosing and last for 30-90 minutes. In the first 3 weeks of the study, the HD group consumed approximately half of the amount eaten by the other groups. The records of food consumption did not show differences between the control, LD and MD groups.

Weight: LD and MD rabbits gained more weight than the control animals. HD dams gained less.

Reviewer's Summary of body weight changes (p.279)

Dose group	Δ from GD1 to GD29 in grams	% difference from control
0	274	
6 mg/kg	378	38
45 mg/kg	456	66
150 mg/kg	185	-32

Mortality: One MD and 3 HD females died within 2 minutes of dosing, possibly from incorrect intubation. Two HD animals died for whom pregnancy status was unknown. The data from these two animals was excluded from all summaries.

Abortions: 1 control, 1 LD, 3 HD females

Summary of maternal indices showing effects

	Dose (mg/kg/day of RS-43285)			
	0	6	45	150
No. females inseminated	20	20	20	20
No. females died prior to scheduled sacrifice	1	1	2	6
No. pregnant	0	0	1	1
No. not pregnant	0	0	1	0
No. pregnancies not determined	0	0	0	2
No. of females aborted	1	1	0	3
No. pregnant at term	13	14	14	13
Implantation index (implantation's/corpora lutea)	87.7	73.8	64.3*	58.4**

significantly different from the controls by the Mann-Whitney test at *p=0.05 and **p=0.01

Summary: Excessive maternal toxicity produced an inadequate number of litters for evaluation by contemporary standards. It may be seen in the existing data that in the absence of maternal toxicity, there was no NOEL for the observation of decreased implantations.

Study title: Oral perinatal and postnatal reproduction study in rats with RS-43285-193

Key study findings: The methods state that only healthy animals were used in the study and then asserts that 1 LD dam was euthanized for health problems apparent before the start of dosing. There was no apparent maternal toxicity. General condition for pups on PN day 1 was recorded in error for approximately 40% of the litters. No data was presented for developmental landmarks. The study is inadequate.

Study no.: 10-R-88-43285-193-PO-PP/AT4822
Volume #, and page #: vol 22, p.54
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: February 11, 1988
GLP compliance: hard to tell. There was a QA statement but no GLP statement
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: LD: ATSA239F; MD: AT342 SA320D; HD: AT342 SA223J
Formulation/vehicle: water, adjusted to pH=4

Methods: Female CD rats (Crl:CD®BR:VAF/Plus) with evidence of mating were randomly assigned to 4 groups of 28 females each. The rats were given 0 (water control), 10, 40 or 200 mg/kg/day by oral gavage from GD15 to weaning at PN21. Litters were culled on PN 1 to 8 pups of 4 of each sex where possible. PN days 4,7,14 and 21, the litters were observed for weight number live and sex.

Results: No compound related signs were reported for any animals. One LD dam was euthanized due to poor health before dosing. One control and 1 HD dam were euthanized due to loss of all pups in their litters. There was no significant difference in average weight gain from post-mating day 1 to post-mating day 29 between the treatment groups.

General condition of pups on PN 1 was recorded in error for approximately 40% of the litters. The sponsor stated that this did not impact the "study conduct or interpretations; the data are not presented in this report."
There were no differences in the data as reported for the gestation and neonatal indices. There were no significant differences in pup weight or survival for either sex. No data was presented for developmental indices. The pups that died in the first 4 days were examined for anomalies. The data was not presented in such a way as to be able to determine the litter incidence.

Summary: The methods state that only healthy animals were used in the study and then asserts that 1 LD dam was euthanized for health problems apparent before the start of dosing. There was no apparent maternal toxicity. Observations on general condition for PN 1 for approximately 40% of the litters were recorded in error. No data was presented for developmental landmarks. The study provides little information as to the post-natal effects of the drug.

Reproductive and developmental toxicology summary:
It was communicated to the sponsor after the filing meeting that the reproductive toxicity studies were inadequate due to the disease outbreak in the fertility study and in other studies due to excessive mortality resulting in insufficient numbers for comparison. The sponsor replied in writing that they disagreed with this interpretation and in their opinion, the studies were adequate and interpretable. This reply is part of the record for this NDA. Based upon the evidence available in the studies the following interpretations were reached.

Fertility: For reasons detailed in the review it must be concluded that drug-treatment caused a profound decrease in male rat fertility that was not alleviated with a drug-free recovery period. Decreased implantation (NS) was noted in female rats and a statistically significant dose-related decrease in implantation index was also reported in the rabbit Seg II study. No data was provided on female cyclicity.

Teratogenicity: The rat study was inadequate by contemporary standards but the sponsor argues that the study is acceptable by the 1966 standards in place at the time of conduct. Dose-related skeletal malformations were reported at all doses, even without maternal toxicity in the rats: LD 5 mg/kg(0.066x human exposure), MD 40 mg/kg (1.14 x human exposure) and HD 400 mg/kg (no data available for estimation). A skeletal effect was apparent in the surviving HD litters of rabbits. However, in this study, the effect may well have been due to maternal toxicity. Exposure relative to humans can only be estimated by surface area comparisons: Rabbit HD of 400 mg/kg ÷3= 133 x37=4933 mg/m^2HED. The MRHD in terms of surface area is 1200 mg/m^2. Therefore, 4933 ÷1200 = 4.1X MRHD on the basis of surface area.

Better selection of doses might have better demonstrated a dose-response effect. As plasma levels were not provided, it is difficult to estimate the relative human exposure. What is troubling to the reviewer is how in the EMLD toxicology studies, a single oral dose of 250 mg/kg caused 40% mortality and a single oral dose of 500 mg/kg caused 60% mortality. The three-month rat study showed 30% mortality in females dosed with 500 mg/kg/day. The rat teratology study also showed approximately 30% maternal mortality at the HD of 400 mg/kg as did the rabbit study (1/20 control, 1/20 LD, 2/20 MD and 6/20HD). Why were the studies started with doses known to produce such severe effects?

Development: Two studies in rats examined post-natal development. The combination fertility-post-natal development study used an insensitive method of assessing developmental landmarks. Despite this, a dose-related delay in negative geotaxis was reported. Delays were also noted in eye opening, incisor eruption and vaginal opening although these effects did not show a dose-response relationship. The second development study had several problems: General condition of the pups on PN1 was reportedly recorded in error for 40% of the litters. The methods state that only healthy animals were used, but 1 dam assigned to the LD group was euthanized for health problems that had been apparent prior to the start of dosing. The reliability of the report is a moot point as no data whatsoever was reported for developmental landmarks, simply a statement that there were no effects. Results of the first study must then predominate and it must be concluded that the drug caused developmental delays at doses with no apparent maternal toxicity.

Overall summary: The sponsor feels that the studies were done to meet the standards existing at the time of conduct and are therefore adequate. The reviewer has stated points of concern in detail in the various reviews. As requested by the sponsor, these studies have been interpreted based upon the data presented.

Reproductive and developmental toxicology conclusions: The drug causes a decrease in male fertility, is embryotoxic, caused an increase in spontaneous bone defects (sternum) and causes developmental delays in the absence of maternal toxicity.

Labeling recommendations: "Carcinogenesis, Mutagenesis, Impairment of Fertility" the sponsor's statement should be changed to read that ranolazine has shown detrimental effects upon male fertility at doses of 300 mg/kg/day (1800 mg/m^2 or 1.5 times the MRHD on a surface area basis). There is unsufficient data regarding cyclicity in females. Ranolazine showed dose dependent embryotoxicity in rabbits at doses of 6 m/kg (74 mg/m^2, 0.06XMRHD), 45(555mg/m^2 0.5X MRHD)and 150 mg/kg(1850 mg/m^2, 1.5x MRHD).

"Pregnancy—Category C" The sponsor's first two sentences in this section should be removed and replaced with a statement that in rats, there is evidence of increased spontaneous bone defects (primarily sternebral) at doses 5mg/kg (30 mg/m^2 or 0.03X MRHD), 40 mg/kg (247 mg/m^2 or 0.2XMRHD) and 400 mg/kg/day (2467 mg/m^2 or 2X MRHD).

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: RS 43285 RQT(3): Acute adrenal function study in rats

Key study findings: After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively.

Study no: AT4437, SS/072/88
Volume #, and page #: vol 22, p 5.

Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: December, 1987
GLP compliance:
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle:

Methods: Male Sprague-Dawley rats (Crl:CD(SD)BR) were assigned to 2 groups of 10 rats each. One group received the vehicle of water, the other group received 300 mg/kg ranolazine. The rats were given 2 doses of ranolazine within a 24 hour period. The rats received the second dose approximately 2 hours prior to blood sampling. Stress tests and euthanasia were conducted between 10.00 and 12.00 hours when then sponsor states that ACTH and corticosterone levels are considered to show least variation due to diurnal rhythm. Each rat was stressed by placing it for 2 minutes in a hollow perspex tube on 2 occasions 20 minutes apart. The animals were then euthanized by decapitation 20 minutes after being placed in the perspex tube for the second time. The blood samples were then analyzed for ACTH and corticosterone. The adrenal glands were weighed. The left adrenal was then assayed for pregnenolone, progesterone, corticosterone and aldosterone. The right adrenal was collected for histopathology.

RS 43285 ROT(3) : Adrenal Function Study (Acute) in Rat

Plasma Results

Dose mg/kg/day		ACTH pg/ml	Corticosterone ng/ml
0	Mean	54.2	255.8
	SD	25.8	95.1
	Mean	48.5	173.3*
	SD	7.9	31.5
		8.54	31.77

SED - Standard error of the difference between means

Statistical significance of differences from the control based on 2-sided t-tests.

p less than 0.05

Results: Plasma ACTH and corticosterone levels were decreased in the drug-treated animals. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone were decreased by statistically significant amounts.

Sponsor's summary of adrenal tissue results

Dose Mg/kg	Weight (mg)	Pregnenolone Ng/gland	Progesterone Ng/gland	Corticosterone Ng/gland	Aldosterone Ng/gland
0	18.7±3.6	80.7±23.8	171.0±70.9	742.6±415.4	6.36±2.19

300	19.6±2.5	56.0*±15.5 (31%)	69.0*±40.1 (60%)	152.0**±114.3 (80%)	2.38**±0.68 (63%)
Sed		9.12	26.07	136.4	0.73

Sed= standard error of the difference between means
Statistical significance of differences from the control based on 2-sided t-tests: *p<0.05, **p<0.01, ***p<0.001
Numbers in parentheses are reviewer's calculation of percent difference from the control value.
After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively.

Study title: RS 43285 RQT(2): Acute adrenal function study in rats

Key study findings: From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia), clinical chemistry or histopathology of the lymphoid organs. From the data presented it can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine.

Study no: AT4436 SS/071/88
Volume #, and page #: volume 21, p 266
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: November 1987
GLP compliance: statement included
QA reports: yes (x) no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle:

Methods: Following sham dosing for 16-18 days, Ranolazine was given twice within 24 hours at doses of 0 and 300 mg/kg to groups of 24 male Sprague-Dawley (Crl:CD(SD)BR, Charles River, UK) rats. Each group was divided into 3 subgroups. The measurements made for each sub-group are shown in the sponsor's summary below.

	Sub-groups		
	1(pre-stress)	2(2 min post-stress)	3 (20 min post-stress)
Plasma ACTH	+	+	-
Plasma corticosterone	+	-	+
Adrenal corticosterone	+	-	+
Plasma lipids/glucose	+	+	+

Total and free cholesterol, triglycerides, non-esterified fatty acids and glucose were assayed in all rats. Stress tests and euthanasia were performed between 10.00 and 11.20 hours to minimize diurnal variation in ACTH and corticosterone. Each rat was restrained for one minute in a

perspex tube to induce stress. It was killed 2 minutes after first being put in the tube and blood for plasma ACTH determination was collected. Adrenal glands were collected and weighed. The left adrenal gland from subgroups 1 and 3 were individually homogenized and lipid extracts assayed for corticosterone content. The adrenals from subgroup 2 were frozen.

Results: The sponsor's summary tables are shown below.

	Dose mg/kg/day	Plasma ACTH pg/ml	Corticosterone Plasma ng/ml	Corticosterone Adrenal ng/gland	Corticosterone Adrenal ng/mg
Basal	0	24.2	4.8	76.3	4.90
	300	21.8	3.0*	34.8*	2.35*
After Stress	0	95.7	235.7	1090.9	73.16
	300	71.6	177.4	444.5**	26.13*
Selr		0.17	0.20	0.27	0.27
Selr (AS)			0.21	0.28	0.28

Selr - Standard error of ln (ratio) between basal groups.

Selr (AS) - Standard error of ln (ratio) between stressed groups.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01

Group Mean Plasma Lipids and Glucose Results

	Dose mg/kg/day	Cholesterol Total mg/dl	Cholesterol Free mg/dl	Cholesterol Ester mg/dl	Triglyceride mg/dl	NEFA mEq/L	Glucose mg/dl
Basal	0	82.6	19.4	63.3	74.3	0.241	163.7
	300	87.9	21.0	66.9	91.6*	0.265	164.6
2 min After Stress	0	78.6	19.8	58.9	120.0	0.329	158.7
	300	100.5**	24.3	76.3**	82.3***	0.195	173.4
20 min After Stress	0	88.7	22.7	66.0	109.3	0.397	164.1
	300	101.0	25.5	75.5	81.8*	0.349	174.1
S.E.D.			2.94				8.0
S.E.D (20)			3.05				8.3
Selr		0.079		0.075	0.104	0.249	
Selr (20)		0.082		0.078	0.107	0.258	

S.E.D. - Standard error of the difference between means
S.E.D.(20) - Standard error of the difference between groups 20 minutes after stress.

Selr - Standard error of ln (ratio) between any two groups.
Selr (20) - Standard error of ln (ratio) between groups 20 minutes after stress.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01
*** p less than 0.001

	Dose mg/kg/day	Plasma ACTH pg/ml	Corticosterone Plasma ng/ml	Corticosterone Adrenal ng/gland	Adrenal ng/mg
Basal	0	24.2	4.8	76.3	4.90
	300	21.8	3.0*	34.8*	2.35*
After Stress	0	95.7	235.7	1090.9	73.16
	300	71.6	177.4	444.5**	26.13*
Selr		0.17	0.20	0.27	0.27
Selr (AS)			0.21	0.28	0.28

Selr - Standard error of ln (ratio) between basal groups.

Selr (AS) - Standard error of ln (ratio) between stressed groups.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01

It may be seen that after dosing with drug, but before being stressed, the ranolazine animals had slightly lower "basal" ACTH and lower plasma and tissue corticosterone levels. After the stressor, plasma and tissue levels of all the above-mentioned parameters increased. However, the drug-treated animals did not show the same mean magnitude of increase as did the control group. Unfortunately, the results shown did not include the standard deviations of the means. We do not know what variability was inherent in the assay or in the animals and thus do not know if the values shown are real or fall within the variability of the assay and lab. Looking at the plasma lipid values, it may be seen that the basal levels of the two groups are essentially the same with the exception of slightly higher triglyceride levels in the drug-treated group. Was 2 minutes post-stress sufficiently long enough to see the full magnitude of response? At both time points after the stressor, the cholesterol levels were higher in the treated vs control groups. Triglyceride levels were somewhat lower. Again, standard deviations were not shown.

Summary
From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia) or histopathology. It can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine. According to Goodman and Gilman (9^{th} edition), opioid receptor binding can cause a decrease in ACTH secretion. Drolet et. al. also noted (Prog Neuro-Psychopharmacol &Biol Psychiat 2001, vol 25, pp729-741) that opioids can diminish stress-induced neuroendocrine and autonomic responses and may stimulate these effector systems in the non-stressed state.

Study title: RS 43285 RQT: One month adrenal function study in rats

Key study findings: There was little difference between the treatment groups in terms of basal plasma ACTH and corticosterone. Following a defined stress, plasma and adrenal corticosterone increased to a greater extent in all drug-treated animals. Only the HD group showed slight increases both in serum cholesterol and triglycerides and in adrenal weight.

Study no: AT4372 SS/061/88
Volume #, and page #: vol 21, p. 164
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 8, 1987
GLP compliance:
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle: water

Methods: Ranolazine was given orally to groups of n=20 male Sprague-Dawley (Crl:CD(SD)BR)rats at doses of 0, 5, 50 and 300 mg/kg/day for 29 days. At the end of this time,

plasma and tissue (adrenal) assays for corticosterone and lipids were performed as well as plasma ACTH. EM and light microscopy examination of adrenal tissue was also carried out. All rats were euthanized 2 hours after dosing. The first 10 rats per group were used to evaluate basal parameters. The last 10 rats at each dose were subjected to stress stimuli (restraint in a perspex tube) and euthanized within 20 minutes of being placed in the tube to evaluate physiologic markers of stress.

Results: Basal ACTH and corticosterone did not differ significantly between groups and given the apparent variability in the measurements, did not differ at all.

Summary of basal plasma ACTH and Corticosterone

Dose mg/kg/day	ACTH pg/ml	Corticosterone ng/ml
0	28±10	21±29
5	39±32	5±9
50	38±17	15±24
300	34±8	10±11

N=10 per group. Numbers are mean ±SD

Following the stressor, plasma corticosterone was increased in both control and treated animals. The increase in ranolazine-treated rats was slightly greater than that seen in control rats.

Summary of adrenal function tests: stress induced corticosterone

Dose mg/kg/day	Plasma Ng/ml	Adrenal		
		Weight mg	Ng/gland	Ng/mg
0	92±32	24±2	844±853	36±36
5	128±59	25±4	1181±648	46*±23
50	163±130	25±4	1395±900	55*±32
300	191±96	35±4	1869±960	53*±26

*$p<0.05$

The sponsor's data on plasma lipids indicates little difference if any between the stressed an unstressed states when one compares within a given treatment group. The drug-treated animals had slightly higher cholesterol levels than the controls. Given the normal range of cholesterol levels that may be found in rats, the results may also fall within the range of normal variability.

RS 43285 RQT : One Month Adrenal Function Study in Rats

Group Summary - Plasma Lipids

Unstressed

	Dose	---- Cholesterol ---- Total	Free	Ester	Triglyceride	NEFA
	mg/kg/day	mg/dl	mg/dl	mg/dl	mg/dl	mEq/L
Mean	0	83	18	65	120	0.18
SD		16	5	12	27	0.06
Mean	5	90	20	70	133	0.20
SD		9	3	7	28	0.08
Mean	50	86	18	69	111	0.36**
SD		13	3	11	30	0.20
Mean	300	111***	26***	85***	93**	0.22
SD		17	5	14	22	0.11

Stressed

	Dose	---- Cholesterol ---- Total	Free	Ester	Triglyceride	NEFA
	mg/kg/day	mg/dl	mg/dl	mg/dl	mg/dl	mEq/L
Mean	0	88	24	64	114	0.19
SD		15	3	15	24	0.10
Mean	5	90	26	64	119	0.31*
SD		16	4	14	19	0.13
Mean	50	100	28*	72	119	0.47***
SD		16	5	12	19	0.21
Mean	300	117***	35***	82***	103	0.63***
SD		15	4	12	21	0.11

* p less than 0.05
** p less than 0.01
*** p less than 0.001
n = 10

The lipids within the adrenal gland show more marked changes at the HD for total, free and esterified cholesterol as well as triglycerides and NEFA.

RS 43285 RQT : One Month Adrenal Function Study in Rats

Group Summary - Adrenal Lipids

	Dose	Cholesterol						Triglyceride		NEFA	
		Total		Free		Ester					
	mg/kg/day	mg/gland	%	mg/gland	%	mg/gland	%	mg/gland	%	mg/gland	%
Mean	0	0.265	1.13	0.064	0.27	0.202	0.86	0.226	0.94	0.020	0.08
SD		0.052	0.22	0.013	0.06	0.047	0.19	0.106	0.36	0.010	0.03
Mean	5	0.284	1.13	0.078	0.30	0.206	0.82	0.260	1.02	0.021	0.08
SD		0.057	0.23	0.018	0.05	0.041	0.19	0.103	0.38	0.010	0.04
Mean	50	0.298	1.21	0.082*	0.33*	0.216	0.88	0.264	1.07	0.020	0.08
SD		0.044	0.16	0.013	0.05	0.035	0.13	0.112	0.47	0.007	0.02
Mean	300	0.581***	1.65***	0.128***	0.37**	0.453***	1.29***	0.380***	1.09	0.043***	0.12**
SD		0.161	0.36	0.054	0.14	0.144	0.33	0.165	0.44	0.017	0.04

* p less than 0.05
** p less than 0.01
*** p less than 0.001
n = 10

The HD group also showed a greater weight of adrenal gland compared to the other treatment groups.

Summary of adrenal organ weights (n=20)

Dose mg/kg/day	Body weight	Absolute adrenal weight	Adrenal weight normalized to body weight [x]
0	359±30	50±7	0.139
5	369±33	52±7	0.141
50	356±42	50±7	0.140
300	371±28	68**±9	0.183

[x]reviewer's calculation from available data

Gross and microscopic observations are summarized below. Changes in the zona fasciculata were most pronounced in the HD group.
Summary of gross and microscopic adrenal changes

	Dose group (mg/kg/day)			
	0	5	50	300
Adrenal enlargement	1/20	1/20	2/20	3/20
Adrenal pallor	1/20	1/20	3/20	14/20
Adrenal discoloration	0/20	0/20	0/20	1/20
Zona fasciculata cytoplasmic vacuolation	6/20	6/20	7/20	16/19
Zona fasciculata cytoplasmic foaminess				
Minimal	7	6	7	3
Slight	11	11	10	10
Moderate	2	3	3	6
marked	0	0	0	19

Electron microscopy changes were reported to include an increase in the number and size of intracytoplasmic membrane-bound vesicles, some so large that the nucleus was compressed. Fusion between vesicles was reported to happen commonly. Some contained a homogeneous, lipid-like substance but more were reported to have a fine, floccular nature. Mitochondria were reported to be enlarged, some cavitated and some containing a flocculent substance. Fusion with the vesicles was also noted. Increased numbers of lysosomes were also reported in the drug-treated animals. The sponsor interpreted the results as evidence of enhanced cellular activity, possibly a normal secretory process.

Study title:RS-43285: Investigative study in rat adrenal cells in-vitro

Key study findings: Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone.

Study no: AT6435
Volume #, and page #: vol 27, p.318
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: February 2, 1993
GLP compliance: statement included
QA reports: yes (x) no ():
Drug, lot #, radiolabel, and % purity: RS-43285 lot E9-ML-001
Formulation/vehicle:

Methods: Adrenal glands were harvested from 2 male Sprague-Dawley (Crl:CD(SD)BR) rats and monolayer cultures prepared. After 2 days of incubation at 37ºC, the culture medium was removed and replicates of 4 wells were incubated with the following:

- Control medium
- 10^{-9}M ACTH ± 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}M RS-43285
- 2.5x10^{-6}M pregnenolone± 10^{-4} RS-43285
- 2.5x10^{-6}M progesterone ± 10^{-4}M RS-43285
- 2.5x10^{-6}M deoxycorticosterone (DOC) ± 10^{-4} M RS-43285

All incubations were for 2 hours except for DOC, which was 20 minutes.The report stated that the incubation times had been previously shown to allow linear kinetics to ensure that enyzme inhibition would be fully expressed. Corticosterone and cAMP were measured with radio-immunoassays.

Results: RS-43285 at concentrations of 10^{-7}M and 10^{-6}M had no effect on corticosterone secretion from ACTH-treated cells. At 10^{-5}M and 10^{-4}M ranolazine decreased corticosterone secretion from ACTH-treated cells by 15% and 87% respectively. Cyclic AMP values were shown only for the ACTH-treated cells and 1 concentration of ranolazine. The sponsor's data is shown below.

Mean Corticosterone and Cyclic AMP results (n=4)

TABLE 1

Treatment group	Medium 199 with	Corticosterone secreted (pmol/well±SEM)	Cyclic AMP secreted (fmol/well±SEM)
A	No additions	0.8 ± 0.27	5.5 ± 0.6
B	10^{-9}M ACTH	11.8 ± 0.65	263 ± 26
C	10^{-9}M ACTH, 10^{-7}M RS-43285	11.6 ± 0.97	-
D	10^{-9}M ACTH, 10^{-6}M RS-43285	14.1 ± 1.55	-
E	10^{-9}M ACTH, 10^{-5}M RS-43285	10.0 ± 0.77	-
F	10^{-9}M ACTH, 10^{-4}M RS-43285	1.5 ± 0.22	426 ± 39
G	2.5x10^{-6}M Pregnenolone	25.5 ± 1.90	-
H	2.5x10^{-6}M Pregnenolone, 10^{-4}M RS-43285	24.8 ± 1.30	-
I	2.5x10^{-6}M Progesterone	25.5 ± 2.20	-
J	2.5x10^{-6}M Progesterone, 10^{-4}M RS-43285	22.1 ± 1.41	-
K	2.5x10^{-6}M Deoxycorticosterone	34.0 ± 1.56	-
L	2.5x10^{-6}M Deoxycorticosterone,10^{-4}M RS-43285	29.8 ± 3.25	-

Under the conditions of the assay, ranolazine added to cells treated with ACTH produced a decrease in detected corticosterone.

Study title:RS-43285: Investigative study in rat adrenal cells in-vitro

Key study findings: Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone.

Study no: CL5566, SS/141/90
Volume #, and page #: vol 35, p. 333
Conducting laboratory and location: Professors Vinson and Laird, St Bartholomew's Hospital, London and Syntex Research
Date of study initiation: february 1988 to March 1989
GLP compliance: no
QA reports: yes () no (x):
Drug, lot #, radiolabel, and % purity: E6 M1001
Formulation/vehicle: Krebs-bicarbonate Ringer solution

Methods: A series of in vitro studies were commissioned from Professor Vinson and carried out as pilot investigations. The primary objectives of the studies were:

a) to assess whether ranolazine directly inhibited coricosteroid synthesis in rat adrenal tissue
b) to assess whether similar effects occurred in dog and human adrenal tissue
c) to investigate sites of corticosteroid synthesis inhibition in rat adrenal tissue.

The results of the effects on rat, dog and human adrenal tissue and the site of inhibition studies were presented. Since the time of conduct of those studies, Syntex developed in house expertise and developed an alternative interpretation of the studies. That interpretation did not concur with Professor Vinson's interpretation. Therefore, that alternative interpretation was also presented in the report. It was noted that additional experiments had been commissioned, were ongoing at Western General Hospital in Edinburgh and would be reported elsewhere.

Rat adreanl tissues were obtained from female Wistar rats (~2 months old). Suspensions of adrenal zona glomerulosa and fasciculata/reticularis cells were prepared by established methods.

Human adrenal tissue was prepared from renal transplant donors.

Dog adrenal tissue was obtianed from 2 male Beagles (~8 and 9 months old).

Four series of experiments were performed:

a) Effect of Rs-43285 on steroid secretion by rat adrenocortical cells: RS-43285 was added to incubations of rat adrenal zona glomerulosa and inner zone cells in the presence or absence of a maximally stimulating dose of ACTH(10^{-9}mol/l). Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l. Control incubations of rat adrenal zona glomerulosa and inner zone cells containing either no additives or ACTH alone were also included.
b) Effect of RS-43285 on steroid secretion by dog adrenocortical cells. RS-43285 was added to incubations of dog adrenal cells in the presence and absence of ACTH 10^{-9}mol/l. Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 5 x10^{-7}, 10^{-6}, 5x10^{-6}, 10^{-5}, 5x10^{-5}, and 10^{-4} mol/l. Control preparations with no ACTH or additives were also prepared.

c) Effect of RS-43285 on steroid secretion by human adrenocortical cells. RS-43285 was added to incubations of human adrenal cells ±ACTH (10^{-9}mol/l). Doses of RS-43285 were 10-8, 10-7, 10-6, 10-5 and 10-4 mol/l. Control incubations of human cells with no ACTH or other additives were also included.
d) Effect of RS88597 on steroid secretion by rat adrenal cells. RS-88597 was added to incubations of rat adrenal zona glomerulosa and inner zone cells ± ACTH(10^{-9}mol/l). Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l. Control incubations of rat adrenal zona glomerulosa and inner zone cells containing either no additives or ACTH alone were also included.

Steroid analysis: The sponsor assayed the major corticosteroid in the chosen species. Corticosterone, aldosterone (all experiments) and cortisol (dog and human only) were measured by radioimmunoassay. 18-hydroxycorticosterone and 18-hydroxycorticosterone were measured by gas liquid chromatography after derivitization.

Results

Rat adrenal zona glomerulosa cells: aldosterone secretion was concentration-dependently decreased from ACTH-stimulated cells with little effect at concentrations $<10^{-7}$. Basal aldosterone secretion was minimally affected at the highest concentration although the graphical presentation is difficult to assess quantitatively.
Significant decreases were seen at 10^{-5}($p<0.01$) and 10^{-4} mol/l ($p<0.001$). ACTH-stimulated corticosterone-release was also decreased at concentrations of $>10^{-6}$ mol/l. There was a spurious decrease at 10^{-8}mol/l. Effects were significant at 10^{-5} ($p<0.01$) and 10^{-4}($p<0.001$) mol/l.
Basal corticosterone was significantly ($p<0.05$) decreased at the highest concentration and appears to show a moderate decrease at the next lower concentration.

Rat zona fasciculata cells: ACTH-stimulated corticosterone was variabily decreased at all concentrations $\geq 10^{-8}$ mol/l with no solid dose-response apparent. The highest concentration of ranolazine produced a slight but significant ($p<0.05$) decrease in ACTH-stimulated 18-OH-DOC secretion. Basal 18-OH-DOC secretion was decreased at concentrations of 10^{-7}, 10^{-5} and 10^{-4} mol/l ($p<0.01$ for each) but with no apparent dose-response. There was a decrease in basal corticosterone secretion at concentrations $\geq 10^{-8}$ mol/l with no concentration response.

Dog adrenal cells: RS-43285 showed a concentration-dependent decrease in ACTH-stimulated release of both aldosterone and cortisol. The effective concentrations were $\geq 5 \times 10^{-7}$mol/l with a flat line at the 3 concentrations $\geq 10^{-5}$mol/l. ACTH-stimulated corticosterone release was also concentration-dependently decreased at concentrations $\geq 10^{-6}$ mol/l. Basal aldosterone and cortisol secretion were also decreased but with little difference in effect between the concentrations from 10^{-6} and 5×10^{-5} mol/l. Basal corticosterone was decreased at concentrations $\geq 10^{-6}$ mol/l with significant decreases $\geq 5 \times 10^{-6}$ mol/l.

Human adrenal cells: ACTH-stimulated aldosterone and cortisol was concentration-dependently decreased at $\geq 10^{-6}$ mol/l. The effect was significant at 10^{-5} and 10^{-4} mol/l with $p<0.001$ for both concentrations. Corticosterone was decreased at concentrations of 10^{-5} (NS) and 10^{-4} ($p<0.001$).

Basal aldosterone and corticosterone release were concentration-dependently decreased at $\geq 10^{-6}$ mol/l. Basal cortisol secretion showed a lesser decrease at the same concentrations.

The effects on 18-)H-β showed a great deal of variability. There were significant ($p<0.01$) decreases in ACTH-stimulated levels at 10^{-5} and 10^{-4} mol/l but no clear concentration-response was evident. Secretion of 18-OH-DOC was significantly ($p<0.001$) decreased at the 2 highest concentrations with essentially no visible effect at the lower concentrations.

As the sponsor states regarding RS88597: The compound decreased ACTH stimulated aldosterone and 18-OH-β secretion by rat adrenal zona glomerulosa (AZG) cells at concentrations of 10^{-5} and 10^{-4} mol/l. Corticosterone and 18-OH-DOC secretion by rat AZG cells was decreased only by the highest concentration of the drug. Basal aldosterone and 18-OH-DOC secretion by AZG cells was decreased by the highest concentration of compound only while corticosterone secretion was decreased by RS-88597 at concentrations of 10-5 and 10-4 mol/l. Rs-88597 also decreased ACTH-stimulated corticosterone secretion by rat adrenal inner zone cells at a concentration of 10^{-4} mol/l. ACTh-stimulated 18-OH-DOC secretion was decreased at 10^{-4} and 10^{-5} mol/l. There was no effect on basal corticosterone or 18-OH-Doc secretion by rat adrenal inner zone cells.

This reviewer concurs with the interpretation of the person who conducted the study and found that:

> Taken together these results show that RS-43285 inhibits both basal and ACTH-stimulated steroid secretion by rat, dog and human adrenocortical cells at concentrations ≥10-5 mol/l. Some effects are also seen at the lower concentrations…It is unlikely that the apparent inhibition at the lower concentration, which occurred when no effect was observed at 10-6 mol/l in the same experiment is attributable to the action of the compound. The metabolite of RS-43285, RS-88597 may be slightly less potent than RS-43285 itself. The maximum concentration of this drug to affect steroid secretion was 10-5 mol/l and in some experiments no effect was seen.

The sponsor of the studies disagreed with this interpretation for the stated reasons that (quoted directly from p. 337, vol 35)

1. The experimental systems used suffer by comparison with those of other laboratories.
2. The sponsor considered the response to ACTH poor and the baseline corticosteroid high which "Consequently, the latitude for convincingly demonstrating inhibitory effects is more limited in Professor Vinson's system than in others."
3. The sponsor felt that the rat zona fasciculata/reticularis cells did not produce a credible dose response.
4. The sponsor felt that the dog adrenal cells showed an IC50 for inhibition of corticosterone synthesis between 10^{-6} and $5x10^{-6}$M and that the difference in the extent of inhibtion between cortisol synthesis (~35%) and corticosterone synthesis (~90%) was difficult to explain.

Overall the sponsor agrees that the results indicate inhibition of corticosterone/cortisol synthesis in vitro. They question whether this was a specific enzymatic effect or whether it was due to cytotoxicity.

Second Set of Studies Within This Report

Adrenal tissue was obtained from female Wistar rats. RS-43285 at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l was added to incubations of rat adrenocortical cells in the presence and absence of added

- 22-hydroxycholesterol (cytochrome P-450 side chain cleavage precursor)
- pregnenolone (3-hydroxysteroid dehydrogenase Δ4-5 isomerase precursor)
- progesterone (21-hydroxylase precursor)
- deoxycorticosterone (11β-hydroxylase precursor)

10 nmol of each precursor was added. Control incubations containing no RS43285 were also included for each added precursor. It was anticipated that significant amounts of deoxycorticosterone would be formed so the analysis for this compound was included. Corticosterone and aldosterone were measured by radioimmunoassay. Deoxycorticosterone and 18-hydroxydeoxy-corticosterone (18-OH-DOC) were measured by gas-liquid chromatography.

Results:
RS-43285 caused a dose-dependent decrease in the secretion of corticosterone and aldosterone by a mixed population of rat adrenocortical cells. The decrease was significant at concentrations of 10^{-5} and 10^{-4} mol/l. A small non-significant decrease in 18-OH-DOC secretion was seen at these concentrations also. DOC secretion was increased by addition of ranolazine at all concentrations. The addition of precursors increased the secretion of steroids.

Added 22-hydroxycholesterol: ranolazine significantly inhibited the secretion of deoxycorticosterone and aldosterone at concentrations of 10^{-5} and 10^{-4} mol/l while corticosterone was decreased by RS-43285 at 10^{-4}mol/l.

Added pregnenolone: aldosterone and corticosterone secretion was decreased at ranolazine concentrations of 10^{-4}mol/l while DOC secretion was decreased by 10^{-5} mol/l but not at 10^{-4} mol/l. There was a non-significant decrease in 18-OH-DOC secretion at 10^{-4} mol/l.

Added progesterone: ranolazine decreased aldosterone secretion at a concentration of 10^{-4} mol/l and coricosterone at 10^{-7}, 10^{-5} and 10^{-4} mol/l but not at 10^{-6} mol/l. A concentration response was not seen for corticosterone. Neither 18-OH-DOC nor DOC secretion were affected.

Added DOC: Only 18-OH-DOC secretion was decreased at the highest concentration of 10^{-4} mol/l.

Basal secretion of corticosterone, aldosterone and to some extent 18-OH-DOC were decreased by added ranolazine. Steroid secretion in the presence of added precursors was also decreased by the addition of ranolazine. The sponsor felt that the results of this study "…should be discounted as suitable substrate concentrations and incubation times were not determined for

each precursor(p. 338, vol 35)". The conducting laboratory addressed this in their conclusion saying that arbitrary amounts of precursors were added and that too great an amount may have overcome the inhibitory effects of ranolazine. Further experiments using a range of concentrations would be necessary to investigate this further. Suboptimal concentrations is one possible explanation for the variability of results and the differences in effect of RS-43285 on steroid secretion. The conducting laboratory suggested other possibilities also. For example:

It may be that the drug is acting non-specifically in vitro with the enzymes to produce the observed effects but the major site of action is at a step in the pathway prior to the conversion of 22-hydroxycholesterol to pregnenolone (such as the hydroxylation of cholesterol, catalyzed by CYP450). Steroid biosynthesis is also controlled by the delivery of cholesterol to CYP450 and that this delivery of cholesterol is increased by ACTH. Ranolazine may interact with this multi-step system.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: There are areas where the pre-clinical database could be strengthened.

General Toxicology Issues: What is the contribution, if any, of the major human metabolites to the adverse effects seen? Will reproductive toxicology studies that meet current standards confirm and even extend the observed reproductive and developmental liability? What is the long term effect, if any, of ranolazine on the pigmented structures of the eye where it and/or a metabolite persist(s) with a half-life of approximately 8 days?

Recommendations:There are several pre-clinical areas that would benefit from further elucidation.

1) The capacity of ranolazine and several of the major human metabolites to bind to cardiac ion channels requires investigation as to potential for causing repolarization abnormalities. The effects upon cardiac contractility and total peripheral resistance seen in the cardiovascular safety study also require further investigation.

2) Clarification of how the sponsor eliminated the various possible mechanisms of action to come to the conclusion that partial inhibition of fatty acid oxidation is the primary mechanism.

3)There are several metabolites that are found in humans at concentrations ≥1%. Systematic characterization of the pharmacology/toxicology of these metabolites would improve the characterization of ranolazine. At a minimum, the metabolites of interest could be characterized in a standard receptor binding screen. Safety pharmacology studies would also be a beginning in the determination of which metabolites are pharmacologically or toxicologically active.

4)The questions regarding the potential reproductive toxicology could be addressed with a combined fertility-development study (such as was submitted), but this time using a HD that does not cause the severe toxicity seen in the existing studies and conforming to the current guidances. A thorough investigation of the potential fertility effects would include but not be limited to: adequate histopathology of both males and females (following the current guidelines from the Society for Toxicologic Pathology), assessment of sperm motility, morphology and number, data regarding the cyclicity of

females and adequate description of developmental parameters using a sensitive detection method.
5)Plasma levels and distribution of drug in the pregnant female and the fetus as well as partitioning into the fetus are a vital part of the pre-clinical characterization.
6)The melanin binding properties of ranolazine and potential effects upon the pigmented structures of the eye require elucidation.

Labeling with basis for findings: see above

X. APPENDIX/ATTACHMENTS:

Addendum to review:

Other relevant materials (Studies not reviewed, appended consults, etc.):

- Studies Not Reviewed:
- The identification of the metabolites of ranolazine in human plasma CL6943
- In vitro metabolism of ranolazine by human liver microsomes and identification of major human cytochrome P450 isozymes involved in the hepatic metabolism of ranolazine CVT303.009-N
- The P170-glycoprotein transporter as a potential site of the ranolazine/digoxin drug interaction. CVT303.010-N
- Determination of the potential inhibitory effects of commonly prescribed drugs on the metabolism of ranolazine by human liver microsomes in vitro. CVT303.011-N
- AT3237:The effects of RS-43285 on the Electrocardiographic Changes Produced by Transient Myocardial Ischaemia in the Dog
- AT 3238: The Effects of RS- 43285 on Regional Myocardial Blood Flow Changes Produced by Transient Myocardial Ischaemia in the Dog
- AT 3239: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Atria to Isoprenaline
- AT 3240: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Ileum to Acetylcholine and Histamine
- AT 3241: Affinity of RS- 43285 for M1 and M2 Muscarinic Subtypes
- AT 3242: In Vitro Affinity of RS- 43285 for Alpha- Adrenoceptors
- AT 3243: In Vitro Affinity of RS- 43285 for Beta- Adrenoceptors
- AT 3244: In Vitro Affinity of RS- 43285 for 5HT -Receptors
- AT 3245: The Effect of RS- 43285 on the Cardiovascular Haemodynamics of the Chloralose Anaesthetised Cat
- AT 3246: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Tracheal Strip to Isoprenaline
- AT 3247: The Effect of RS- 43285 on High Affinity Uptake of Noradrenaline and 5-Hydroxytryptamine into Brain Synaptosomes
- AT 3249: Affinity of RS- 43285 for D1 and D2 Dopamine Receptors of Rat Striatal Membranes

- AT 3250: The Effect of RS- 43285 on Nicotinic Responses to Acetylcholine in the Frog Rectus Abdominis Muscle
- AT 3251: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Atria to Histamine
- AT 3252: Effects of RS- 43285 on Sodium Currents in the Guinea- Pig Myocardium AT 3253: The Effect of RS- 43285 on Adenosine Binding to Rat Frontal Cortex
- AT 3254: The Effects of RS- 43285 on Intra- Atrial, Atrio- Ventricular and Intra- Ventricular Conduction in the Isolated Langendorff Perfused Rabbit Heart
- AT 3258: Possible Involvement of Myocardial Adenylate Cyclase in the Action of RS- 43285
- AT 3261: The Effects of RS- 43285 on the General Haemodynamic Changes Produced by Transient Myocardial Ischaemia in the Dog

- AT 3267: Calcium Entry Blocking Effects of RS- 43285 in the Guinea- Pig Myocardium

- AT 3284: Effects of RS- 43285 on Potassium- Induced Contractures of Guinea- Pig Mesenteric Artery, Portal Vein and Porcine Coronary Artery

- AT 3285: Effects of RS- 43285 on Calcium- Dependent Slow Action Potentials Recorded from the Guinea- Pig Myocardium
- AT 3286: Effects of RS- 43285 on Active Potassium Currents and Membrane Permeability of the Guinea- Pig Myocardium
- AT 4708: The Effects of Ranolazine on the Ischaemia- Induced Increase in Alpha1- Adrenoceptor Density in the Rat Left Ventricle
- AT 4765: Effect of Ranolazine on Reperfusion- induced Cardiac Fibrillation and Survival After Various Periods of Ventricular Ischemia in Working Rat Hearts in Vitro
- AT 4766: Effect of Oral and Intravenous Ranolazine on Coronary Arterial Ligation and Reperfusion- induced Cardiovascular Morbidity and Mortality in Anaesthetised Rats
- AT 4828: The Effect of RS- 43285- 193 and Its Two Enantiomers (RS- 43285- 197 and RS- 43285- 198) on Rat Cardiac Cyclic AMP Phosphodiesterase
- AT 4991: The Effects of Ranolazine on the Blood Pressure Response to Haemorrhagic Hypovolaemia in the Anaesthetised Rat
- AT 5032: Effects of Ranolazine (RS- 43285) on the Energy Metabolism of the Rat Isolated Heart Subjected to a Period of Temporary Ischemia: Study Using Phosphorus- 31 NMR Spectroscopy
- AT 5298: Effect of Propranolol or Ranolazine Infused Alone or Together as a Mixture on the Performance of Rat Working Hearts Subjected to Left Ventricular Ischaemia and Reperfusion
- AT 5299: On the Role of Oxygen- Derived Free Radicals in the Production of Ischaemia and Reperfusion Injury and Ranolazine's Protective Effect in Working Rat Hearts In Vitro
- AT 5304: Effects of Ranolazine on Ouabain- Induced Toxicity in the Guinea- Pig Papillary Muscle Preparation
- AT 5307: The Action of Ranolazine at Thromboxane (TP) Receptors in Isolated Guinea Pig Aorta
- AT 5425: Effects of RS- 43285- 193 on Guinea- Pig Cardiac Ventricular Action Potentials "In Vitro" under Normal and Ischaemic Conditions

- AT 5450: The Binding of [3H]- RS- 43285- 193 to Rat Cardiac Mitochondria
- AT 5458: Comparison of Vasodilatation in Rat Aorta Evoked by Sodium Nitroprusside and RS- 43285- 193
- AT 5712: Reduction of Myocardial Enzyme Release by RS- 43285 (Ranolazine) in a Subhuman Primate Model of Ischaemia with Reperfusion: Post- Reperfusion Treatment with RS- 43285- 193 (racemate) and its Enantiomers RS- 43285- 197 (S- isomer) and RS- 43285- 198 (R- isomer)
- AT 5713: Limitation of Myocardial Enzyme Release by Ranolazine (RS- 43285- 193) in a Subhuman Primate Model of Ischaemia with Reperfusion: Pre- Ischaemia Treatment with RS- 43285- 193
- AT 5714: The Effect of RS- 43285- 193 on Adenosine Uptake Sites in Guinea- Pig Brain
- AT 5787: Effects of Ranolazine (RS- 43285- 193) and Nitrendipine in a Rat Model of Calcinosis
- AT 5800: The Effects of Ranolazine on Organ Preservation in Porcine Renal Autotransplantation

- AT 5993: Effects of Ranolazine on Inotropic Responses in the Guinea- Pig Papillary Muscle Preparation
- AT 6130: The Effect of Ranolazine on Myocardial Infarct Size in a Canine Model of Regional Myocardial Ischaemia and Reperfusion. Estimation of the Degree of Infarct Size Reduction.
- AT 6488: Test of RS- 43285- 193 (Ranolazine) for Inhibition of Lipid Peroxidation in Primary Rat Blood Monocytes (RBM) Using the Fluorescent Polyunsaturated Fatty Acid, Cis- Parinaric Acid
- AT 6632: Effects of Ranolazine and its R- and S- isomers on the Consequences of Coronary Artery Ligation of Rat Hearts In Vitro and In Vivo
- AT 6733: Measurement of Plasma Ranolazine and Myocardial Enzyme Markers in Samples from Lucchesi Dog Infarct Study
- AT 7001: Effects of Ranolazine on Positive Inotropic Responses to Forskolin and High Stimulation Intensity in the Electrically- Paced Guinea- Pig Papillary Muscle Preparation
- AT 7006: Effects on Ranolazine on L- Type Calcium Channel Currents in Single Guinea-Pig Ventricular Myocytes
- AT 7007: Further Studies on the Effects of Ranolazine on Metabolic Substrate Utilisation in the Isolated Rat Heart
- AT 7011: Effects of Ranolazine in In Vitro Neurotoxicity Models
- AT 7012: Pilot Studies on the Effects of Ranolazine on High- Energy Phosphate Content and Intracellular pH in Ischaemic Langendorff- Perfused Rat Hearts as Assessed Using 31 P-NMR
- AT 7023: Beneficial Effects of Ranolazine on Porcine Renal Preservation
- AT 7025: Effects of Chronic Ranolazine Treatment on Exercise Performance and Other Parameters in Rats With Myocardial Infarction and Chronic Heart Failure
- AT 7026: Preliminary Studies on the Effects of Ranolazine in a Rat Model of Myocardial Infarcted Congestive Heart Failure
- AT 7039: Protective Effects of Ranolazine on Ventricular Fibrillation Induced by KATP Activation in Isolated Rabbit Hearts During Hypoxia and Re- Oxygenation
- AT 7080: Effects of Ranolazine on Myocardial Metabolism in Anesthetized Swine

- CL 5832: In Vitro Effects of Ranolazine (RS- 43285- 193) on the Uptake and Catabolism of Adenosine in Human Red Blood Cells
- CL 5973: The Lack of Effect of RS- 43285- 193 on the Oxidative Burst of Human Neutrophils
- CL 5985: Ranolazine (RS- 43285), a Putative Anti- ischaemic Agent, Inhibits Complex I of the Mitochondrial Respiratory Chain
- CL 6073: Analysis of Actions of Ranolazine on Hormone Regulated Adenylyl Cyclases CL 6208: Studies on the Effect of Ranolazine on the Cyclic AMP Signal Induction Pathway
- CL 6482: Tests of RS- 43285- 193 (Ranolazine) for Inhibition of Lipid Peroxidation in Human Low Density Lipoproteins Using the Fluorescent Polyunsaturated Fatty Acid, Cis-Parinaric Acid
- CL 7067: Effects of Ranolazine on Membrane Lipid Peroxidation

- CL 7068: Ranolazine Inhibition of Respiratory Complex I: Evidence for Greater Potency in Broken or Uncoupled than in Coupled Mitochondria
- CVT303.023- N: The Effects of Ranolazine on Palmitate and Butyrate Oxidation in the Rat Heart
- CVT303.024- N: A Study of the Effects of Ranolazine on AV Nodal Conduction Time in Guinea Pig, Rat and Mice Hearts Using His- Bundle Electrograms
- CVT303.025- N: Effect of Selected Fatty Acid Oxidation Inhibitors on Palmitate and Glucose Oxidation in Rat Isolated Hearts
- CVT303.023- P: Effects of Ranolazine on Infarct Size Following Regional Ischemia and Reperfusion of the Rat Heart
- CVT303.039- P: Effects of Ranolazine on Action Potentials from Human Ventricular Myocytes
- CVT303.044- P: Effects of Acute Intravenous Ranolazine on Cardiac Function in Dogs With Advanced Heart Failure: A Dose Escalation Study
- CVT303.048- P: Electrophysiologic Effects of Ranolazine in the Guinea- Pig Heart In Vivo
- CVT303.004- R: Studies on the Effect of R, Sand RS Ranolazine on Crotonase Activity

The following studies are for impurities that are either not longer present in the drug product or are present in levels low enough that qualification is not needed.

- 124- 006: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing RS-88056 in Sprague- Dawley Rats
- 124-013: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 2458 (Des- Methoxy Ranolazine) in Sprague- Dawley Rats
- 124-014: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 245.9 (P- Methoxy Ranolazine Isomer) in Sprague- Dawley Rats
- 124- 015: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 2511 (Ortho- Chloro- Des- Methoxy Ranolazine) in Sprague- Dawley Rats
- 124- 016: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 3379 (N- RAN3- RAN) in Sprague- Dawley Rats
- 124- 018: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 4795 (O- RAN3- RAN) in Sprague- Dawley Rats

- 124- 019: 28- Day Repeated Dose Toxicity Study of Ranolazine Free -Base Containing CVT -2728 (Desmethyl Ran) in Sprague- Dawley Rats

Any compliance issues:

APPENDIX II Carcinogenicity Summary

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